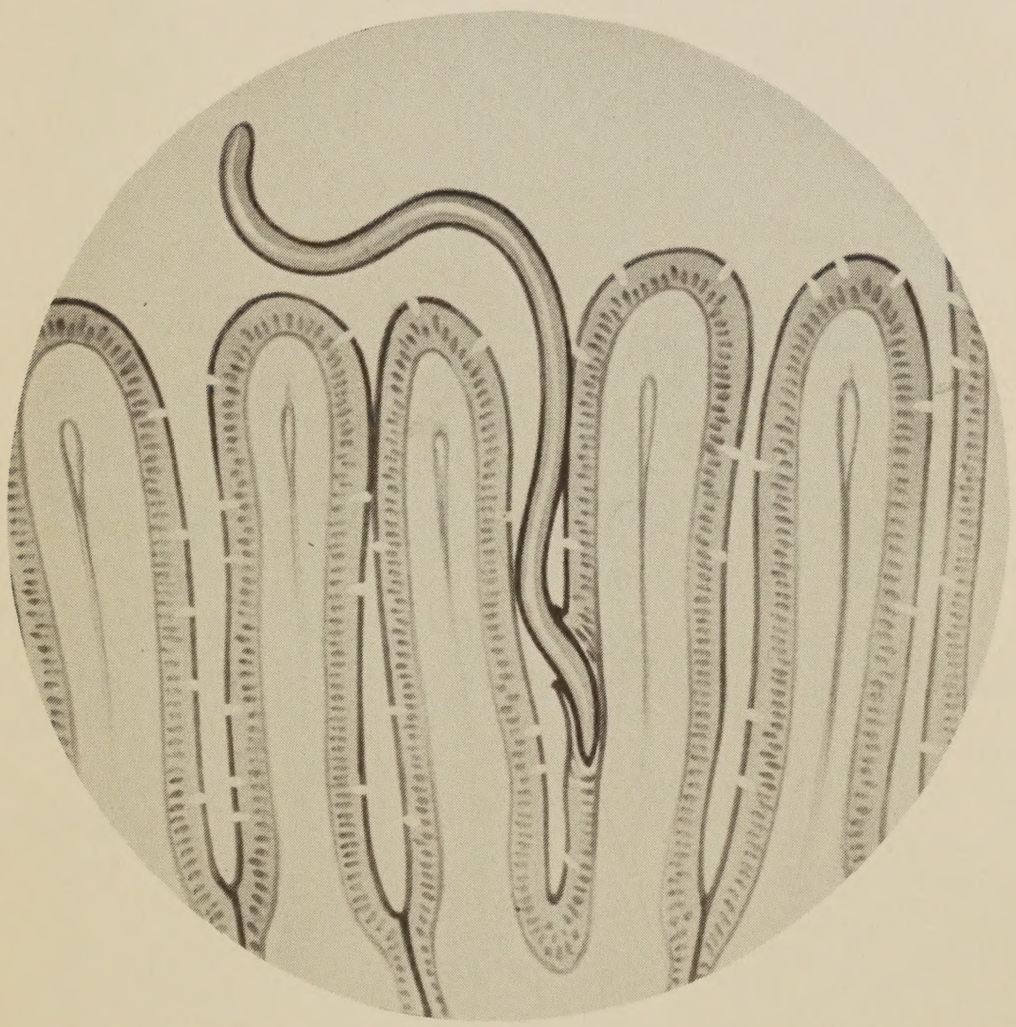
Cues That Influence Behavior of Internal Parasites



CUES THAT INFLUENCE BEHAVIOR OF INTERNAL PARASITES

Proceedings of a Workshop

September 21-23, 1981
Auburn, Alabama

Sponsored by
Regional Parasite Research Laboratory
Agricultural Research Service
U.S. Department of Agriculture

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Agricultural Research Service

Agricultural Research Service
U.S. Department of Agriculture
May 1982

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Cover: L_1 larva of Trichinella spiralis entering its intramulticellular niche within the columnar epithelial cells of the small intestine.

This publication is available from the Regional Parasite Research Laboratory, P.O. Box 952, Auburn, Ala. 36830.

DEDICATION

To the memory of Neil A. Croll, 1941-1981, whose participation in this workshop was prevented by his untimely death on June 23, 1981.

Published by the Agricultural Research Service (Southern Region), U.S. Department of Agriculture, P.O. Box 53326, New Orleans, La. 70153. Issued May 1982.

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FOREWORD

All who have concerned themselves with parasite life cycles have been intrigued by the quite bizarre migratory routes that some parasites follow, with various stages passing through or residing temporarily in tissues of great diversity. Haemonchus has a relatively simple life cycle, yet we do not know why this parasite characteristically develops in the abomasum rather than elsewhere in the gastrointestinal tract of ruminants. For parasites with more complex life cycles, questions about site location, migration, and development are more numerous and perplexing. Consider the migration of Fasciola to the liver, first to the hepatic parenchyma and then into the bile passages, or the migration of Stephanurus to the liver for its early development and then to the perirenal tissue for its maturation--after a tract has been established through which the eggs can pass into the ureter. These examples can be multiplied over and over. Always the question remains: "Why?"

There has been much speculation about but relatively little systematic investigation into the factors responsible for the enigmatic behavior of some parasites. The rapid developments in recent years in physiology, immunology, and molecular biology have made us acutely aware of our ignorance about the complex phenomena which are responsible for parasite behavior. At the same time, these developments have suggested that more effective control of parasitic infections might be achieved through better understanding of the ecology of the parasites in all their stages. When adequate knowledge is available, it may be possible to control internal parasites by removal of or interference with behavioral cues that influence establishment, site location, development, or reproduction. But manipulations that interfere with normal development or behavior of parasites could result in aberrant parasitism, and aberrant parasites often cause more damage to the host than do parasites in their normal location. This is perhaps even more reason for promoting research on behavioral stimuli, for the increasing use of drugs, hormones, vaccines, and other biological and chemical agents in man and animals may disrupt behavioral cues.

This workshop was held to provide an overview of some of the research on this subject. The program was arranged to allow

time for extended informal discussion of each presentation, and an effort has been made to capture the essence of these exchanges. It is hoped that this volume will promote expansion of research on this exciting frontier of parasitology.

W. S. Bailey

GASTROINTESTINAL PHYSIOLOGY: ENVIRONMENTAL FACTORS INFLUENCING INFECTION AND PATHOGENICITY

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INTRODUCTION

The environment of the gastrointestinal (GI) tract is modified by diet, the flow of endogenous secretions and, in certain regions, by the activities of microbes. These factors contribute to a dynamic luminal habitat for parasites in contrast to a more stable one in the tissues making up the wall of the hollow organs.

Specific conditions that influence the ability of a parasite to establish in the GI tract are poorly understood, but would be expected to vary depending on the infective stage and the habitat chosen by postinfective stages. If the infective stage is an egg or cyst, the luminal environment and its modifying factors are of paramount importance in releasing the encased organism. These factors along with those in the unstirred layer of fluid overlying the epithelium are important to the survival and sustenance of emerging noninvasive forms. Invasive organisms are influenced further during their establishment, growth and development by factors associated with epithelial and underlying tissues. Whereas there is no significant evidence to support the concept of radially distributed differences in the environment of the small and large intestines, differences can be found along the length and within the depths of these organs.

Lumen-dwelling parasites interact superficially with the host tissues, generally inducing only mild responses. Tissue invaders, on the other hand, induce more severe reactions, which teleogically, are aimed at eliminating or confining the organisms. Unlike many microorganisms animal parasites do not produce potent toxins. Pathogenesis of disease is related directly to traumatic damage or lysis of tissue through the release of histolytic enzymes, and indirectly through proliferative changes within tissues.

The host response to invasion may involve nonspecific resistance factors manifested by various forms or degrees of acute and chronic inflammation. The latter encompasses reactions leading to granuloma formation, fibrosis, and possibly calcification. The response may also involve specific immune reactions. Eliciting antigens may be derived from somatic tissues of parasites or from their excretions and secretions. Antibodies and hypersensitivity states, although having dubious connections with functional immunity, may play important roles in mediating inflammatory lesions.

Whereas specific conditions that influence infectiveness of parasites in normally susceptible hosts are poorly defined, several factors influence the pathogenesis of disease once an infection is established. These include the (1) type of parasite involved, (2) intensity of infection, (3) duration of infection, which may be influenced by immune state of the host, and (4) unique characteristics of the injured tissue or organ.

The latter factor will be considered in detail, focusing on the structural and functional uniqueness of the GI tract, i.e., the physiology of this system. Emphasis will be on the normal, dynamic state of the GI environment, factors that regulate or alter it, and potential influence of these alterations on parasite infectivity and pathogenicity.

GI TRACT AS A VARIABLE ENVIRONMENT

The adaptive potential of the organs comprising the GI system must be considered to adequately assess whether the magnitude of changes induced by any agent exceeds that consistent with maintenance of homeostasis. An adaptive response is one which allows an animal to better cope with its environment. It is important to note that the GI tract undergoes significant morphological and functional changes in response to relatively innocuous stimuli. An example is the response to food, as alluded to earlier. Feeding or fasting have considerable influence on physiological activities. The extent and duration of changes related to fasting depends in large part on the length of time an animal goes without food.

Effects of Short-Term Food Deprivation

An example of how short-term food deprivation influences the GI tract is seen in intestinal smooth muscle motility patterns as an animal cycles between periods of fasting and feeding. Small intestinal myoelectric activity, an established correlate of motility, has been recorded in dogs from electrodes surgically implanted on the serosa of the small intestine. Using this technique, Schanbacher et al. (1978) have monitored changes in myoelectric activity daily. So-called slow wave activity, represented in graphic tracings by broad, rounded depolarization-repolarization events and spike activity, represented by more

rapid depolarization-repolarization superimposed on slow waves, were observed.

The contractile activity of the small intestine, as percent slow waves with spikes per 2 min recording intervals, during fasted and fed states were measured over 4-h periods. The fasted state began with spike activity on only a few slow waves. The number of slow waves with spikes increased until a period was reached in which all slow waves had superimposed spikes. This pattern stopped abruptly and then repeated itself every 20-30 min, with maximum activity separated by 90-100 min intervals. After feeding, the pattern changed to one in which 20-50% of slow waves had spikes for extended periods without breaks.

Parasites in the small intestine may drastically alter this "normal" fasted-fed pattern of myoelectric activity. This was shown when dogs were infected with the enteric stages of Trichinella spiralis. The infective stage of this nematode is a larva encysted in skeletal muscle. When these infective larvae are ingested by the host they embed in the epithelium of the small intestine (Wright 1979) and develop into mature male and female worms within 36 hours. Within the first week of infection F_2 larvae are deposited by females, and these larvae migrate via the blood to skeletal muscle where they encyst. The initial intestinal phase of a primary infection lasts for about two weeks in the dog before the worms are expelled in association with the development of inflammation, which is, in part, immune-mediated. The muscle phase lasts for the life of the host.

In dogs infected with this parasite, motility changes were seen as early as 18 h postinfection (PI), but were maximal 3-4 days PI. As the infection coursed through the second week, motility returned to the preinfection state. Secondary infection failed to produce significant changes from the control patterns.

These findings are interpreted as follows. Different and distinct patterns of motility are associated with fasting and fed states in the uninfected host. Primary infection eliminated the fasting pattern, giving rise to a prolonged change that resembled a constant feeding pattern. Failure of a challenge infection to evoke similar changes illustrates how prior contact with the parasite, probably through induction of immunity, can modify the effect of parasitism on the host's GI tract.

Effects of Long-term Food Deprivation

The ability of parasites to survive in a changing environment indicates that they are insensitive to changes of the nature reflected by fluctuating motility patterns or have developed mechanisms to cope with the changes. Interestingly, T. spiralis induced, shortly after infection, a pattern of intestinal

motility that was more consistant than that in the uninfected host--a constant fed pattern. Does this change in some way benefit the parasite? The answer to this question is open to speculation.

Considering the other half of the host-parasite system, is the parasite-induced alteration a detriment to the host? Decreased food intake or anorexia and related sequelae are common in trichinellosis. The possibility that motility patterns resulting from infection provide a "signal" that influences host satiety is an issue that bears on the pathogenesis of disease. Also, anorexia and the consequential absence of food in the GI tract may influence the GI environment significantly.

The potential influence of cessation of food intake on interactions between parasites and their host can be partially surmised from studies dealing with rats kept from eating for as long as 2 wk. In these animals both structural and functional changes in the GI tract are extreme. These studies will be discussed focusing on two areas. One concerns changes in GI structure and function that may be interpreted as pathologic. The other concerns possible effects of these changes on infectivity, growth and development of enteric parasites.

Changes in small intestine structure and function. During the time rats were deprived of oral food for 10-14 days they were sustained in positive nitrogen balance by total parenteral nutrition (TPN) using a liquid diet as originally described by Steiger, Vars and Dudrick (1972). In these studies GI parameters associated with parenteral nutrition were determined and compared with these in animals fed the same liquid diet or an isocaloric stock diet orally (Castro et al. 1975).

An obvious change in the small intestine of parenterally nourished rats was a decrease in intestinal weight and small intestine:body weight ratio (Castro et al. 1975). The decrease in intestinal weight was selective, since comparisons of other organ weight:body weight ratios in rats on TPN revealed no differences (Johnson et al. 1975). The drop in total weight was due to a decrease in mucosal as well as muscle mass and was reflected in microscopic changes (Castro et al. 1976). Villus height as well as crypt depth clearly decreased from control values. Although the mucosal weight:gut weight ratio was not significantly altered by TPN, the amount of mucosal tissues per unit length of intestine was reduced (Castro et al. 1976). Gut length remained unchanged. Mucosal dry weight, protein, lipid and carbohydrate content all decreased significantly (Castro et al. 1976).

These changes in tissue mass were associated with changes in both disaccharidase and peroxidase activities. Disaccharidases are important in contact or membrane digestion of dietary di- and oligosaccharides and are localized on the brush border

of epithelial cells. Peroxidase, presumably myeloperoxidase (Castro et al. 1974b), is a lysosomal enzyme involved in antimicrobial systems in certain phagocytic cells. Studies indicate that mucosal peroxidase activity reliably estimates the number of myeloid cells in the lamina propria (Castro et al. 1974b, Smith and Castro 1978). TPN causes a statistically significant reduction in both disaccharidase and peroxidase activities (Castro et al. 1975, 1976).

Influence on parasites in primary infection. The reduction in mucosal mass and peroxidase levels during parenteral alimentation suggested a decrease in the number of inflammatory cells in the lamina propria. This could have a bearing on resistance to infectious agents. There is no information on the ability of patients on prolonged TPN regimens to respond to antigens or biologic agents entering the body via the oral or enteric route, although the ability of such individuals to elicit normal delayed and humoral immune responses when challenged parenterally has been reported (Law et al. 1973, 1974). This problem was examined by conducting studies on the infectivity, growth and development of T. spiralis, a tissue-invading nematode, and Hymenolepis diminuta, a lumen-dwelling tapeworm, in rats on TPN. The infective stage of H. dimunita is a cysticercoid larva found in insects such as fleas and various beetles that have eaten tapeworm eggs. Once ingested the cysticercoid larva develops into a segmented flatworm about 18-20 inches long within 14-17 days. The individual segments of the worm, which are collectively called a strobila, arise from a budding zone or neck region just behind the head or scolex.

Rats on TPN were totally resistant to infection with tapeworms and harbored fewer T. spiralis than orally fed rats, if the hosts were being fed parenterally at the time of exposure to the parasites (Castro et al. 1974a). These results indicated that the absence of food from the GI tract caused changes that interfered with infectivity or the nutrition of these helminths. This presented the possibility that removal of exogenous food from the gut may induce changes that rid an infected animal of parasites or at least decrease the intensity of infection. This was investigated by studying the course of infections when orally fed, parasitized hosts were switched to TPN.

Two groups of rats were infected with either 8×10^3 T. spiralis larvae or 10 tapeworm cysticercoid larvae and then placed on a stock diet and water. Two days after infection with T. spiralis and 16 days after infection with tapeworms, half of the rats in each group were switched to total parenteral nutrition. All T. spiralis-infected animals were killed 5 days later, i.e., 7 days PI. Worms were recovered from the intestine of these animals and various morphological and physiological parameters in the intestine were measured (Castro et al. 1976). The number of adult T. spiralis recovered from

parenterally nourished rats was significantly higher than that recovered from enterally fed controls, but the sex ratios and worm size were not altered significantly. It was not clear why the hosts shifted to parenteral nutrition harbored more worms, but it may be related to the lower peroxidase levels in the mucosa as compared to orally fed rats. Peroxidase activity in the mucosa of conventionally infected rats increases during the intestinal phase of trichinellosis and parallels acute inflammatory cell infiltration of the lamina propria. Immune mediated inflammation is generally accepted as being largely responsible for worm expulsion after a primary infection (see Larsh and Race 1975). From this, it can be surmised that the lower peroxidase levels in parenterally fed rats reflects a decreased inflammatory response which, in turn, might reduce worm expulsion and account for a larger worm burden.

The pathogenesis of intestinal trichinellosis, in parenterally fed rats, as reflected by disaccharidase deficiencies, is similar to that observed in enterally fed hosts. Although brush border enzyme activities in infected rats on TPN were reduced to a greater degree than those in orally fed controls, this was probably due to an additive effect of infection-induced deficiency plus a reduction caused by parenteral feeding.

Some tapeworm-infected rats were killed at the time total parenteral nutrition was initiated and others at various times during a 21 day period of parenteral feeding. When rats harboring gravid tapeworms were placed on TPN the tapeworms destrobilated to the point that after 7 days of intravenous feeding, only worms comprised of a scolex and a very small strobila were found. These worms then underwent secondary proglottization and within 14 days developed into worms with fully developed eggs that were infective to the intermediate host.

Length:weight ratios for tapeworms from enterally fed rats, rats on TPN for various periods, and rats transferred after 7 days of parenteral nutrition back to oral feeding for 14 days reveal two major trends. First, following destrobilization the worms grew steadily to their original length. Second, the length:weight ratio of gravid worms from parenterally fed hosts was greater than gravid worms from both enterally fed rats and rats transferred back to oral feeding.

The length:weight relationship for worms from parenterally fed rats is, in part, a characteristic of a well recognized phenomenon in tapeworm development known as the "crowding effect"--as the number of worms present in the intestine increases, individual worm mass decreases disproportionately to length (Roberts 1961). Judging by the length:weight ratio, tapeworms from hosts on TPN appeared to be "crowded" even though the worm density was low. Despite this small population of worms in the gut, crowding may have been a problem because the gut from parenterally fed hosts was about one-half to one-third the

diameter of enterally fed controls; thus, worms from parenterally fed rats were inhabiting a relatively limited environment.

Influence on parasites in secondary infection. Whereas the previous two studies were concerned solely with host responses to primary infections, another study has revealed the influence of TPN on the anamnestic response of rats to secondary infection with T. spiralis (Castro et al. 1976). The specific aim of this work was to examine the response of immunized rats to a challenge infection administered immediately after an extended period of parenteral nutrition.

Immunity to T. spiralis is partial rather than complete and can be revealed and characterized by following and comparing the course of a primary and secondary infection. The prototype of immunity to T. spiralis which was accepted for many years developed from the work of Larsh and co-workers who studied the immune response in the Swiss albino mouse (see Larsh and Race 1975 for review). In this host, immunity is generated by a primary infection in which worms establish in the small intestine, begin to be expelled by 1 wk and are completely expelled by 14 days. In the secondary or challenge infection worms establish, but survive for a shorter period.

Castro et al. (1976) studied rats that were immunized by a primary infection and fed parenterally for 2 wk. They were then challenged and switched immediately to oral feeding. These rats harbored significantly more worms in their small intestine than did immunized rats that were challenged but maintained on an oral, isocaloric diet throughout the study. Despite the differences in initial worm loads, both groups expelled their parasites by 1 wk PI. These results revealed two points of interest. First, worms were rejected much quicker in the immunized Sprague-Dawley rat than in the Swiss albino mouse. Second, TPN interfered with initial worm rejection. These observations were compatible with the hypothesis that in Sprague-Dawley rats there are at least two phases of immunity to T. spiralis, one which rids the host of parasites very quickly, i.e., reduces the intensity of infection, and another that eliminates parasites that evade the first reaction and become established, i.e., reduces the duration of infection.

Host-parasite interactions. In general, studies on parasitism using TPN to sustain the host should add to our understanding of or at least slant our approach in studying infective processes and pathogenicity of enteric parasites. Despite significant changes in GI structure and function caused by the prolonged absence of food in the intestine, switching animals from intravenous to conventional, oral feeding regimes causes no apparent ill effects. Since specific physiological functions in the GI tract vary considerably with the feeding state, and sick animals tend to decrease food intake or fail to feed at al, it is difficult to dissect those components which may be produced

as part of a primary host response to the parasite from those caused secondarily by reduced food intake. This consideration raises the question: does a measured decrease in a particular physiological parameter indicate an impairment to the health of the host or does it represent an adaptive response on the part of the host to a changing condition?

The prediction of what course an infection with intestinal parasites will follow must take into consideration changes in the host intestinal environment as well as feeding mechanisms, nutritional requirements and the adaptive capacity of the parasite.

The reduced infectivity of T. spiralis for rats on TPN at the time of infection and the augmented "support" of previously established worms by transfer of hosts from oral feeding to TPN can best be explained at this time by alterations in host physiology. There is no evidence of physiologic alterations in the parasite, although they may occur. Trichinella spiralis apparently obtains nutrients from host tissues where it resides.

In contrast to T. spiralis, the reaction of H. diminuta to shifts in the route by which the host is fed is an adaptive response of the parasite. De- and restrobilization and cessation and resumption of egg production as the host is alternated between oral and parenteral nutrition requires the appearance and disappearance of biochemical events out of their normal developmental sequence. Nutrients for secondary growth of H. diminuta following destrobilization must be derived entirely from the exocrino-enteric circulation, a finding which clashes with current concepts regarding tapeworm growth and survival.

The genetic capacity of helminths to acclimate to or escape from environmental change or stress not normally encountered during the life cycle has been defined as exploitative adaptation (see Fairbairn 1970). This adaptation reflects the capacity of H. diminuta to survive in a changing intraintestinal environment.

Destrobilization with retention of scolices as a response to elimination of oral food intake by the host is not a unique finding. Ford (1972) reported this to occur with Hymenolepis citelli in the thirteen-lined ground squirrel as a response to host hibernation. This response was interpreted to impart a definite adaptive advantage with regard to survival of the cestode, since nutritional requirements of the worm would be reduced. Restrobilization of H. citelli occurred only after oral food intake was resumed following arousal of the host. Hymenolepis diminuta in parenterally fed rats underwent subsequent regrowth after destrobilization without resumption of oral feeding by the host.

SUMMARY--NARROWING OF THE ENVIRONMENT

Studies of rats immunized to T. spiralis showed that TPN influenced at least one phase of immunity to the parasite, the early rejection phase. This finding has led to a series of studies which clearly support the hypothesis of a multiphasic response in the immune host. The response which eliminated intestinal worms within 24 hr in orally fed hosts and was suppressed in parenterally fed rats is now known to occur within 15 min after contact of infective larvae with the small intestinal mucosa (Russell and Castro 1979). The rapidly rejected larvae pass through the small intestine in a viable state (Hessel et al. 1982). Further, the rejection process is associated with rapid changes in epithelial cell physiology (Castro et al. 1979, Hessel et al. 1982). These changes include net secretion of fluid and reductions in active uptake or organic solutes and ions in the small intestine. These findings give rise to the hypothesis that worm rejection is not due to direct effects of immune elements on the parasite but to physiologic changes, possibly pathologic in nature, in the epithelial habitat of the worm. Changes in the parasite's microenvironment in immune hosts may be indirectly responsible for poor worm infectivity.

Despite many unanswered questions regarding the phenomenon, the overall problem illustrates the expanding or, conversely, the narrowing of one's view of the intestinal environment and the influence of environmental factors on parasite infectivity and pathogenicity. It shows that one's views of the GI environment may be as variable as the environment itself.

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DISCUSSION

HERLICH: Gil, you said that worms are already starting to be kicked out by the challenge in 15 minutes. Where are the cysts of the challenge dose in 15 minutes; aren't they up in the stomach?

CASTRO: Yes, normally. I didn't go into that. That's a problem when we're looking at a 24-hour response. The idea was to determine how early rejection occurs, so we tried to go from a 24-hour response back down to time zero to figure out how soon we would see worm rejection, again looking at the number of worms that actually established as an endpoint. If you get down to six or eight hours after you orally feed an animal by stomach intubation and try to recover the worms from the intestine, you can find some; but you also have many larvae staying in the stomach. They begin to dribble into the intestine at uncontrolled rates. We couldn't go under six hours and obtain accurate counts. What we did to get answers to the question was to take the animals and implant catheters into the duodenum with the opposite end opening to the exterior, then excyst the worms in vitro and drop them right into the duodenum. So we obviated the problem of gastric emptying time regulating worm distribution. The worms go into the intestine very quickly. If they don't penetrate quickly they will be expelled due to normal peristaltic activity. If one puts a marker like Chromium 51 in the intestine through these catheters, it has already reached the caecum in about 20 minutes. The larvae are not setting up shop as early as 15 minutes post-challenge.

Our present technique does not permit us to find out exactly how soon they penetrate. Maybe they are not going in at all. That's the other possibility. Right now we can't determine whether they actually fail to penetrate the mucosa or penetrate the epithelial cell and are expelled by changes which take place. We think that the epithelial cell is doing more than blocking larval entry because functional changes are occurring in the epithelium after challenge. This indicates that the epithelial cells are actively involved in the rejection response. We are now going to examine changes that we can measure in epithelial cells very quickly after challenge that could be associated with worm rejection. If a blocking-type reaction is involved, then we're thinking IgA or other immunoglobulin on the surface may play a role; but then we have to account for the fact that the epithelial cell can respond too. What kind of feedback system do you have between surface antibodies and epithelial cells to explain altered functions?

DAMIAN: I was interested to see that your approach of challenging on top of the feeding manipulation led you to the conclusion of multiphasic immunity, the same conclusion reached by people like McGregor and Bell using a more traditional type of manipulation. I suppose your approach would tend to bias

you toward non-specific and perhaps they are more biased toward specific effects. I understand your interest in the host-parasite interplay.

CASTRO: I'm obviously biased, but in the direction opposite to what you might expect. I think that there is a very specific response. I'll give you my bias right now. I think it might be more unconventional than the ideas McGregor and Bell may have. Because of the rapidity of the host response, because of the epithelial cell functionally coming into play very quickly, I think we might be looking at the epithelial cell as an effecter cell in this immune system. Maybe it is not only an effecter cell but one possessing a trigger mechanism too. There's memory that may be associated with the epithelial cell.

DAMIAN: I seem to recall a report--perhaps from Hugh Miller or his lab--of influence by some of these cells on mucous cells. Do you envision such a tie-up in the epithelial cell system?

CASTRO: Yes. This is Miller and Nawa's work that you are referring to, where they showed that they could transfer an acquired influence on goblet cells. Basically what they were looking at was Nippostrongylus infection with a catarrhal type inflammatory reaction with increased numbers of goblet cells and mucous secretion. They found they could transfer this response. They could decrease the time it took to elicit this type of reaction if they took lymphocytes--they used thoracic duct lymphocytes--and transferred these from an animal that was already infected with Nippostrongylus to a non-infected recipient and then gave a primary infection after cell transfer. They could show very clearly that the goblet cell numbers increased dramatically, much faster than would occur if that animal had not received the cells.

With regard to your question, there is a tie-in, but again this is a long-term effect that you are thinking of. We're not dealing with events occurring within minutes as is the case with Trichinella. This is a long-term effect with "Nippo." I don't know how lymphocytes could elicit the reaction that could cause rapid rejection in trichinosis. I have asked people this but haven't gotten an answer that satisfies me. I think lymphocytes could be involved in priming the host to do that, but how they could trigger the system, I don't know. I think the reaction is more in line with an IgE type of response, or possibly an IgA--some way unconventionally tied in with a feedback system to the epithelial cells. Something we don't know is whether IgA may function inside the cell rather than having to be secreted. Could IgA be a specific receptor for a particular antigen either incorporated into the membrane or intercellular with a response occurring when the antigen comes in contact with it? Remember that it has to elicit rapid feedback.

DAMIAN: Why does it have to be IgA?

CASTRO: It doesn't. It could be IgE. It could be any immunoglobulin--but inside the cell. Could it work inside the cell with a parasite?

DAMIAN: It could transduce the signal.

CASTRO: What I'm saying is that we don't know now. This is an important question, because we don't know what is happening to that worm. Does it only have to contact the cell membrane or must it penetrate before it elicits the reaction?

DAMIAN: I have one final question. In the shrinking of the gut that you have demonstrated, have you studied the immune elements? What happens to the plasma cell and so forth?

CASTRO: We haven't looked at that.

DAMIAN: I'm wondering if that's something that depends on the route of nutrition.

CASTRO: It may well go locally. People who are fed intravenously for long periods of time respond normally to such antigens. We don't know what's happening at the level of the gut, whether what you're talking about is affecting the G.I. tract. We haven't looked at that, and have no information specifically except for the effect with Trichinella.

FAYER: Along those lines, have you or others looked at the effects of feeding something that's nondigestible to an animal that's fed I.V. and has the gut changes? In other words, would the mere physical presence of something passing through the gut keep it at the "fed level" and maintain or restore functioning?

CASTRO: Yes. If you allow the animal to drink that liquid diet instead of receiving it I.V., the parameters that I showed you would be unaltered from the normal because of the presence of the liquid diet in the gut. Feeding chow or solids would do the same thing. One control that we use in these studies is allowing the animal to drink the liquid diet.

FAYER: When this is done, the cells are functioning for absorption and so forth. I'm asking if the mechanical presence of some non-digestible material in the diet of animals fed I.V. is adequate to keep them at the "fed level," or do the cells have to be functioning and absorbing and so forth?

CASTRO: We haven't done that in parenterally-fed animals and I don't know of anyone who has, but these changes and these parameters can be altered by bulk, just bulk in the diet. One of the things that I didn't mention is that in these rats the presence of food in the intestine will have a great effect on

the release of gastrointestinal hormones like gastrin, which is one of the hormones that control acid secretion. If you put food in the intestine, the circulating gastrin levels go up. If you feed rats the liquid diet that we're using you can maintain most of the intestinal parameters at normal levels, the ones that I have mentioned, but this will not release gastrin. The gastrin level will go down to almost zero. So the composition of diet is important in controlling function.

FAYER: That's an entirely different ballgame than diet composition.

CASTRO: Composition could play a very important role.

DESPOMMIER: I have a few questions and comments. I would like to mention why, at least from my standpoint, the thinking revolves around secretion--and that is the work that we did back at McGregor's school before and after Robin got there. That was to isolate thoracic duct lymphocytes which had Ig on their surface. We would percolate them down through beads which had anti-Ig attached to them. Once they stuck to the column, a lot of cells in the population turned out to have IgA on their surface; and about 30 percent of these cells we found homing in the gut, not in Peyer's patches, where I suppose some people have looked. IgG-producing cells are there also, and I think some IgG secretion goes on as well--but not as much as IgA. I think that's one of the things to be considered with regard to the immunity. But there are so many other inflammatory changes. Everybody is confused now!

CASTRO: Let's get back to the question Ray asked. That was a loaded question in terms of the view about specificity. Somewhere you have a specific trigger system that elicits nonspecific reactions. I guess all that we have done is to gear our thinking to consider all of this as taking place at the level of the epithelial cells--i.e., both specific and nonspecific reactions in the overall mechanism.

DESPOMMIER: Now to return to that trigger mechanism. You don't get 100 percent rejection of worms. About five to ten percent of worms that "take" are rejected after seven to ten days. It depends on the genetics of that particular host. Do you think that the rapid expulsion of the portion of the dose that doesn't take is triggered by the smaller proportion of worms that take? Is that what initiates a rapid rejection response?

CASTRO: No, I don't; and I'll tell you why. We've done some experiments to try and figure out what causes that small worm population to stay there--that is, fail to be rejected. One of the ideas that we had was that maybe we're looking at a genetic variant of the worm, a variant that can bypass that initial rapid-rejection process. That's not the situation based on the

evidence that we have. I can tell you about an experiment which ruled out the possibility of a genetic variant. The other possibility is that we're dealing with something which we call the antigen threshold. This means that it takes a certain number of worms to latch onto the mucosa--five percent of the population that will produce enough antigen to trigger rapid rejection. With the dose that we use we can get anywhere from 50 worms up to maybe 300 worms taking in immune rats, whereas in a primary infection one expects 2,000. That is a big difference. So the idea behind the antigen threshold hypothesis is that we should be able to implant most worms in populations below 300 in an immunized animal. They should all take if you are below that threshold. So we used 10 worms, 25 worms, and 50 worms. Even at these small doses it's still a small percentage of the worm population that takes--about ten percent. So the small percentage of worms taking after our standard dose is not not what's triggering rapid rejection. What we are leaning towards now is that there must be a certain percentage of soft spots in the intestine that are not primed to reject and maybe the worms are just randomly picking out these spots; in other words, a certain percentage of the worms will find a certain number of spots. We have a way of testing this idea.

Let's get back to the questions revolving around the reason why the small population of worms does stick in the mucosa of immune hosts, again thinking about the fact that it might be due to a genetic variation in the worm population. I will tell you how we ruled this out. We would infect animals, leave them for 24 hours and treat them with methyridine. We did that three times. Very solid immunity developed; it's rapid rejection. This is elicited without having the F_2 generation of larvae established in the muscles. When you challenge the animal and have that residual population sticking in the gut, what you can do is collect their progeny. If you collect the muscle larvae and build up this population by serial passage and transfer them back into an immunized animal, you don't get a greater than normal infection. It's the same old worm with regard to infectivity.

DESPOMMIER: Have you tried to establish the infection further down in the gut in a rapid-rejecting animal? The work of Sukhdeo and Croll indicates that wherever you put the worm to begin with, that's where they're going to stay. I'll talk about that tomorrow. This is a summary of their results and obviously not mine. Let's assume for the moment that there are some soft spots in the small intestine which are permissive. Of course, it's non-permissive in terms of rapid rejection. One would assume that the ability of the gut to respond immunologically wanes as you move toward the large intestine, which I believe we'll all agree happens. What about in the large intestine? For instance, have you tried to go directly there? I think you did some serial studies on the gut before; they were very interesting in terms of changes.

CASTRO: Based on the work that you cited, plus the work of people who have looked at the influence of genetics on worm distribution patterns, it is true that these patterns vary. The worms tend to squeeze into the proximal half of the intestine to a large degree in Sprague-Dawley rats. In some hosts, worms are more equally distributed along the gut. What controls that? We're not real sure; I'm certainly not sure. Intestinal parameters that change, such as motility and secretion, may be important. The secretory effect or motility changes may be aimed at trying to wash out or push out the worms. We have looked at this, trying to mimic changes that we see. We tried to mimic these using drugs of one type or another to slow down or speed up motility and to cause or inhibit secretion.

Motility can be gauged by looking at gut transit, which can be measured in rats by implanting a cutaneous-duodenal catheter, putting a marker down it to see how far this marker moves in a given test period. Let's say that we take an animal that is immunized (been infected once) and block transit with morphine. This just stops anything from moving down the gut. There are contractions, but of a non-propulsive segmental type. Nothing will flow through when these animals are injected with morphine sulfate. Based on the studies we're doing now with these rats that have electrodes implanted, we know we are getting some motility changes quickly. Whether they are repulsive or not is another question. We know we can stop the propulsive activity in the gut with morphine. If the animal is immunized and you superimpose an infection on top of the action of the drug, worms can be kept in the gut for several hours, depending on how much drug you use. Those worms will stay in the lumen of the gut. They will not penetrate; even though they have all this time to penetrate, they're not going to go into the mucosa. If you perfuse the intestine, you will wash out all those worms except that residual population that has embedded. So it's not a matter of time that is necessary for penetration.

In terms of secretion, when we challenge these animals, we're finding net secretory changes, the accumulation of fluid in the lumen. The idea we had was to try and mimic this effect. We have been successful in causing the gut to secrete at the same level in a non-immunized rat as we see in an immunized rat after challenge. We've tried agents like prostaglandins and vasoactive intestinal peptide, a secretion-inducing peptide. The most consistent agent that we found was a hypertonic buffer solution. We can use a hypertonic solution to induce the degree of secretion that we see in immunized, challenged animals and even go above that. And it doesn't take too much hypertonicity, compared to say 290 or 300 milliosmolar solution which would be isotonic with blood. We used solutions to 350 milliosmolar. You can get the gut to secrete much more than it would in a rejection response. If you go too high, you can damage the gut irreversibly. If you superimpose an infection

on the maximally secreting gut, the worms still stick in the mucosa in a non-immunized animal. All we are saying is that in the immunized animal we see a certain level of secretion which we mimic in the non-immunized animal. If secretion is responsible for rejection, we ought to be able to get rejection in a non-immunized host that is induced to secrete. But that's not what happens; even when you load up the gut with a hypertonic solution and cause maximal secretion, those worms still embed in that gut.

DAMIAN: Based on what you just said, I'll make this suggestion for an experiment I think would be an equally good test of whether the gut is permissive or whether there are certain populations of worms that are capable of overcoming this. If you give 3,000 worms to Sprague-Dawley rats and have 300 worms resulting from that infection and rapid rejection, and then kill the rat and take those 300 worms and try to infect another rat, would you not be then able to determine whether 300 worms or 30 are established from the latter infection? That would be the test of the hypothesis. If you got 300 worms attaching in the second infection, then it's a special population of worms. If you got 30 worms attaching, then it's a proportion of all the worms that are there.

CASTRO: I've already told you we got the answer to that question.

DAMIAN: I'm sorry. What did you do? You did that experiment?

CASTRO: Yes, with the antigen-threshold experiment, plus looking at two generations of established worms. I think your experiment is a good one, but it would not provide us with a different answer. I think we have evidence to tell us that we shouldn't have to worry about this antigen threshold but that we probably have those "soft spots" in the gut. I'm just saying that there are areas of the intestine which are not primed for rapid rejection. We're saying that the fact that we get a certain percentage of worms indicates that there must be habitats or microhabitats in the gut that are "safe" for the worm in the immunized animal. For one reason or another there is a defect in the rejection system at these sites.

FAYER: But no one has defined those anatomically.

CASTRO: No, we're just guessing--because we're trying to get at the question that Dick asked, "What are the factors that allow this certain percentage of worms to go into the gut of an immunized animal--to override that rapid rejection response?" It takes a second mechanism of immunity to get rid of those worms that do establish. Why do they go in to begin with?

FAYER: If there are those soft spots, then your opening statement is incorrect, that there's no radial difference.

CASTRO: There may well be. I said that there's no evidence; however, we must still rule out that they are not distributed longitudinally.

METTRICK: As usual you have covered the waterfront in your presentation. I've got three or four comments and questions in no particular order--one with regard to those changes in enzyme levels which you showed. The intestinal mucosa, as you know, does have very considerable flexibility in changing the enzymes being secreted. It's just a device by the animal to insure that unwanted enzymes are not produced, that they only produce the enzymes that are required. When you go to the parenteral feeding and you're not putting any fluid in the gut, it would be expected that those enzyme levels normally would be very drastically reduced. This is what you showed. But I don't see why peroxidase increased. Do you have an explanation for that?

CASTRO: The peroxidase would not be elevated. It was reduced in the parenterally-fed animal, primarily because of the decrease of the mass of the tissue, but also because there is a decrease in specific activity. Overall mucosal mass is decreased. If you superimpose the infection on top of the parenteral feeding, then you will raise the level from the basal activity, which is really sub-basal, because you're dropping it by the parenteral nutrition. When you infect, you raise it. But you're not raising it to the level that you would get if that animal had been on oral nutrition. That was the point.

METTRICK: Also, under normal circumstances there is efflux of glucose from the mucosal cells during parenteral feeding. In a normal animal--not an infected animal--that accounts for between 15 to 18 percent of luminal glucose content.

CASTRO: How much of that is total glucose?

METTRICK: Well, we don't know. The point is that this continual glucose is coming from luminal secretions. There is also the reverse taking place. The glucose is being leaked out or diffused out. Now, I can envision a situation with those very small worms in contact with the mucosa. But they could, in fact, use or have access to some glucose which had leaked at a low level. But the worms then later on grow to the same length. What I'm wondering is whether these worms have a capability similar to that of humans. If you go on starvation, you will first of all start to utilize the carbohydrates. That obviously means proteins start breaking down; then the glucose from those proteins is used to build the essential sugars required. It's intriguing to wonder whether these worms have these capacities, perhaps under those circumstances, to do essentially what we do under starvation conditions, which might lead to that rather stringy, long worm that you have.

CASTRO: Getting into adaptive responses by a parasite is intriguing from the standpoint of metabolism of that organism. Again, it's a carbohydrate-utilizing organism to begin with. All the work that came out of Fairbairn's lab in the '60's and '70's shows that 30 percent of the weight of Hymenolepis diminuta is lipids. A good portion of that is triglycerides or glycerides in general; these organisms do not have the capacity to utilize those lipids. It's a tremendous energy reserve and no one knows their function. It would be interesting to study "adapted worms" to see if they do possess the capacity to utilize these lipid stores in light of the fact that they are not getting the normal complement of carbohydrates coming into the gut.

Did I mention the work of Ford? This whole idea of destrobilization and restrobilization is not new. It's certainly not new with us; Benny Ford (1972) published in Experimental Parasitology that he had studied destrobilization, using Hymenolepis citelli in the 13-lined ground squirrel. In this host he could induce hibernation. He would get destrobilization of the worm during host hibernation. Those worms would remain as a scolex until the host was aroused and starting feeding again. The difference between Ford's work and ours is that we have a host that is not hibernating but one in which the food source is rerouted. Here the worm destrobilates but will restrobilate without host refeeding it, so that is different. The destrobilization in hibernating animals is an adaptive response--a shedding of all this extra worm mass that's taking up nutrients in the gut. Nutrients are limited, but the minute amounts present are enough to get the strobila to survive. In our case it could be that either the worm has adapted to the nutrients that are in the exocrinoenteric circulation or to a way that it can begin to utilize lipids for energy production. It couldn't utilize lipids before it destrobilated. In short, two possibilities exist: the host may be adapting to put more nutrients in the gut to cause the worms to grow in this environment, or the parasite may be doing all the adapting.

METTRICK: Did you measure the amount of microflora, because in animals where the microflora has been drastically reduced, you get the same sort of morphological changes in the gut. Microbiologists have argued that one of the reasons for the present normal gut structure is that it is part of the immunological defense mechanism by the animal to these microorganisms that are in the gut. I was wondering if they had any shifting changes?

CASTRO: I believe what you're saying and think the changes in the direction that you're talking about are obviously real, but we haven't looked at the microflora.

METTRICK: A last comment here. I don't know a lot about immunology. But with the H. diminuta model there now seems to be

some evidence that the immunological response certainly comes into play in this so-called crowding effect. We've recently done some experiments in which we have established H. diminuta infection and then given a normal dose of histamine every day during the entire period. We have found that the number of worms is drastically reduced. The size of the worms has changed; they are longer but thinner, and they have far fewer eggs. Interestingly, if you then take these worms and feed them to beetles, the number of cysticercoids that develop in these beetles is also reduced. I wonder whether you had, in fact, looked at histamine level in these rats.

CASTRO: No, this is getting back to the earlier question about plasma cells. We haven't looked at these parameters at all. If you're saying that maybe immune phenomena are playing a role in the crowding effect, it's my understanding that when this occurs and you clear the crowded population of worms and then reinfect with a non-crowded population, you would then get normal worm development. If it's an immune phenomenon, why wouldn't you see some influence on that new population that is being developed?

METTRICK: Well, I think that's still a controversial point. Hopkin's recent book reviews that sort of thing, but it's still a questionable area.

KLESIUS: Would it be correct to say that with the primary response you have associated some inflammatory change?

CASTRO: In the primary?

KLESIUS: Do you feel like you have dissociated the inflammatory response from the initial rejection mechanism?

CASTRO: This is in the immunized animal?

KLESIUS: Yes.

CASTRO: It depends on what elements of inflammatory reaction you're looking at. If one looks at the influx of myeloid cells, for example, or changes in peroxidase levels at the time that the worm is being rejected, we could certainly say that these factors are not important, because we can't see any cell influx, and we can't see any rising peroxidase level that occurs fast enough to associate with rejection. But there are many of the biochemical events of inflammation that we have not examined. We can't rule those out. I guess it depends on what level you want to consider.

KLESIUS: At least the effector cells would probably be there in the immune animal.

CASTRO: We probably don't have to worry about some kind of

recruitment process. Probably everything that is necessary to reject worms is already in place. I think it's safe to say that.

BENNETT: I was very interested in a point you weren't very terribly confident about. But I picked it out. I think you mentioned that the secondary response to infection may not necessarily involve direct contact of the immature parasite with the surface of the epithelial cells. Is this what you meant to say or suggest?

CASTRO: No, what I was saying is that it may not be a direct effect of the effector system on the worm. In other words, killing the worm--that may not be necesary.

BENNETT: But you do believe there has to be a contact?

CASTRO: Oh, definitely yes; I think there has to be contact. You could say there doesn't have to be contact, based on the observations that we have. You could say that there are factors inside the lumen that are influencing a reaction in some way. But I feel that you do have contact. The question is whether it's contact and prevention of penetration or penetration followed by rejection. That's the question we have at this stage.

METHODS USED TO STUDY THE NERVOUS SYSTEM OF SMALL PARASITIC HELMINTHS

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I would like to begin my talk by referring to a statement which I feel is relatively important with respect to the topic of this symposium. I feel that all behavior can ultimately be traced back to a series of neurochemical and neurophysiological events within the organism of interest. In our lab we have taken the approach of studying the basic neurochemical and neurophysiological mechanisms which regulate the motor and electrical activity of the parasitic trematodes Schistosoma mansoni and Fasciola hepatica. Our approach attempts to emulate the work of Eric Kandel (1976), the prominent neuroscientist who often refers to the concept of the "cellular basis of behavior." Behavior is defined as anything an organism does involving action and response to stimulation. I should mention that in addition to these two trematodes we have recently started projects on the nematodes Litomosoides carnii and the small root-knot nematode from the genus Meloidogyne. First, let me start with a brief overview of the tools we have used to study the neuromuscular system of parasitic helminths, and then I will follow this with a more detailed discussion of their application. I'm not going to present a talk designed to prove a particular hypothesis; rather it is designed to review the state of the art of methods used to study helminth neuromuscular systems.

An overview of the first phase of the basic methods that we have used to identify and localize the major neurotransmitter substances associated with the nervous system of various parasites can be summarized as follows:

1. Histochemical techniques--with these techniques you can tell where neurotransmitters are located, or enzymes associated with neurotransmitters.

2. Biochemical techniques--with these methods you can analyze the nature and determine quantity of neuro-

transmitter present in parasites. In addition, you can determine the presence or absence of a biochemical pathway for a neurotransmitter.

3. Receptor techniques--with these techniques you can identify and characterize receptors (usually macromolecules on membranes) which mediate physiological functions.

With these methods one is basically able to make statements concerning the transmitters that are potentially involved in regulating nervous activity of the parasite and their location. Research which depends solely upon Phase I methods is incomplete without studies directed towards an analysis of the physiological impact of neurotransmitters upon a given parasite. The following are considered as Phase II methods:

1. Physiological analyses of chemicals, drugs, etc. on muscle activity of the parasite. (These are easy to do on large but more difficult on small parasites.)

2. Electrophysiological studies on muscle activity and an analysis of drug action, using:

   (a) suction electrodes (E.E.C. recordings),
   (b) extracellular electrodes, and
   (c) intracellular electrodes.

With these methods we can understand the physiological impact of neurotransmitters and drugs on the motor activity and electrophysiological activity of the nervous system of the parasite.

At present there are three basic techniques for measuring motor activity from small parasites. One developed by Hillman (see Hillman and Senft 1973) at the University of Texas involves measuring motility by using a fiberoptic system which basically measures the worm's activity as it crosses an array of fiberoptic pathways. Another method is that of Brown et al. (1973) in England, and this simply measures the doppler effect produced by worms moving within a medium. The method that we have been using in our lab is one that directly measures muscle activity using a system which I will describe in greater detail. It is important to note that a method must not only measure just the wiggling of the parasite, which the methods referred to above will do, but also measure changes that occur in rates at which muscle tension increases or decreases and certain other subtle phenomena that occur in the muscle that are often very difficult to detect. Another approach that we have been using involves electrophysiological methods. In one such method suction electrodes are used to measure the electroencephalogram or myogram patterns generated from the neuromuscular system of the parasite. The other methods involve the direct measurement of

cellular activity through the use of microelectrodes that are placed outside or inside a cell. Microelectrodes with finer tips are used to measure the electrical characteristics of the membrane of a particular cell.

To be more specific, let me call your attention to work that has been done on Schistosoma using a method for the histochemical localization of the enzyme acetylcholinesterase, which is a very important enzyme in the metabolism of the neurotransmitter acetylcholine. With this method, one can clearly show that acetylcholinesterase of the schistosome is discretely localized within the nervous system (Bueding et al. 1967) of the parasite as it has been defined in previous literature using classical staining techniques to localize neuronal structures within S. mansoni (Looss 1895). One can use this technique to characterize the organ systems that are innervated by the cholinergic nervous system of the parasite. A different technique can be used to localize the catecholamines or monoamines within Schistosoma (Bennett and Bueding 1971) and Fasciola (Bennett and Gianutsos 1977). With this technique a massive innervation of the head region by dopamine and/or norepinephrine neurons of the immature F. hepatica can be seen. Large neurons, containing what appears to be either norepinephrine or dopamine, can be located about 1 mm behind the headganglia of S. mansoni. A catecholamine nerve net can be demonstrated in peripheral tissues of F. hepatica, with a delicate and widespread innervation. There are projections that come from a major catecholamine-containing nerve trunk of S. mansoni that actually proceed out of the deeper tissues of the parasite and project upwards to, or just below, the surface of the schistosome. When one incubates male S. mansoni in a precursor neurotransmitter substance such as 5-hydroxytryptophan (5-HTP), which is decarboxylated to 5-hydroxytryptamine (5-HT) (Bennett and Bueding 1973), numerous little yellow spots appear all over the schistosome's body. Cross sections of these worms reveal fibers that project from the deeper parts of the parasite up to the dorsal and ventral surface. The preincubation of the parasite in 5-HTP appears to amplify or allows one to pick up more clearly the presence of 5-HT-containing neurons in the parasite. Lastly, in terms of histochemical techniques, one can incubate the parasite in very low concentrations of tritiated 5-HT and pick up the tritium in very discrete regions of the parasite. French investigators (Dei Cas et al. 1979) have used autoradiographs to show that the tritium has accumulated in areas of the central ganglia and adjacent to the muscle layer.

We next turn to neurochemical methods and will indicate very briefly what can be done. For example, the distribution and concentration of norepinephrine and dopamine within the adult Fasciola hepatica can be demonstrated. We have identified the neurotransmitters in the various regions of S. mansoni, and their concentration within these regions can be determined (Gianutsos and Bennett 1977).

With respect to receptors, obviously these are very important macromolecules that are responsible for mediating the action of certain chemicals such as neurotransmitters and drugs. Not only will isolation and characterization of these receptors provide vital biochemical data, but they could also be very significant to the immunologist, especially if such a receptor is associated with the surface of the parasite. For example, when the very potent antischistosomal drug (called RO-11-3128) is applied to the parasite, a rapid contraction of its musculature takes place. S. mansoni responds in a dose-related manner to the compound, whereas S. japonicum does not respond to this drug at all (Bennett 1980). As an antischistosomal drug this compound has been found to be effective against S. mansoni but to have no activity against S. japonicum (Stohler 1977). Given this information, we used radiolabelled RO-11-3128 to determine whether or not we could detect the presence of a receptor that would specifically bind to this drug. We observed that the Kd, or disassociation constant, of this compound for S. mansoni is about 10-12 times less than the association constant for S. japonicum, which correlates to some extent to the interaction of the drug with the parasite's musculature (Pax et al. 1978). Further studies on this receptor have indicated that the drug binds specifically to the intact parasite in a stereospecific manner and that only those benzodiazepines which have antischistosomal activity will displace the labelled RO-11-3128. Structurally related compounds which do not have antischistosomal activity will not affect the binding of the drug to the parasite. In further studies we found that a high proportion of the binding sites are located on the epidermis or tegumental fraction of the parasite. Further work on this receptor has been limited by the fact that the receptor is present in very low amounts and thus would require grams of worms to purify even a few micrograms of the receptor.

We next move into what I consider Phase II of our project, which relates to the parasite's nervous system and the problems of how you analyze the effects of compounds on the musculature of such a small parasite as S. mansoni. In our labortory we developed an apparatus a few years ago which is used to record the muscle activity of the parasite and the electrical activity being generated in the tissues of the parasite (Fetterer et al. 1977). Using this instrument, we can demonstrate that if we stretch the schistosome one millimeter the parasite responds by increasing its rate of contraction. We don't know what chemicals mediate the stretch response; we only know that it is present. We have been able to detect a rather interesting action with respect to the effects of the antischistosomal drug praziquantel. In the presence of Hank's balanced salt solution, praziquantel causes a rapid and immediate increase in the tension of the parasite's musculature; basically it goes into a spastic contraction and remains that way in the presence of 10^{-6}M praziquantel. On the other hand, if one applies the drug in the presence of Hank's balanced salt solution

containing a high concentration of magnesium (30 mM) one sees a transient contraction followed by an immediate relaxation. A parallel study using high potassium (60 mM) shows that it too can cause a rapid contraction of the parasite's musculature. Again, by placing the parasite in Hank's balanced salt solution containing high magnesium one can observe that the potassium-induced contraction is not markedly affected by the presence of high magnesium, although one can clearly see that upon addition of praziquantel there is an immediate relaxation of the musculature (Fetterer et al. 1980). This would indicate that praziquantel might be increasing the permeability of the parasite towards magnesium.

In a further series of experiments we wanted to determine whether or not the rate of relaxation induced by praziquantel in the presence of high magnesium was in any way correlated to the concentration of praziquantel in the solution. We have shown that 10^{-7} praziquantel relaxes the muscle rather slowly, whereas an even faster relaxation of the parasite's musculature occurs when the parasite is placed in the drug at a concentration of 3×10^{-7}. Finally at 10^{-6} one can see that the rate of relaxation is even more rapid. Thus, it seems clear that the rate at which the muscle is relaxing in the presence of high magnesium and praziquantel is a function of the concentration of the drug in the bathing media (Bennett, unpublished information). In another series of experiments we demonstrated that a K^+-induced contraction of the parasite's muscle could be blocked by the Ca^{++} antagonist D-600 while a praziquantel-induced contraction was not blocked by D-600. When Mg^{++} was elevated to 30 mM, the K^+-induced contraction could be reversed (i.e., the parasite's muscle would relax) when 10^{-6}M praziquantel was added to the medium. The unique thing about D-600 is that it will block calcium flux through tissues where calcium is allowed to enter by a change induced as a result of a change in the resting membrane potential of the preparation. That is, D-600 blocks voltage-dependent calcium channels which obviously would be the channels that would probably be opened up by application of potassium to the schistosome muscle; i.e., it would affect movement of calcium by changing the muscle membrane potential (Mayer et al. 1972). Since praziquantel-induced contractions of the musculature of S. mansoni are not associated with a depolarization of the parasite's muscle, then one would predict that D-600 would not block praziquantel-induced contraction of the parasite's musculature. In short, praziquantel-induced contractions are not dependent upon a voltage change of the membrane potential, and thus D-600 would not be expected to block the praziquantel-induced contraction.

To complicate things a little bit, it is interesting to note that high magnesium does not block the PZ-induced contraction of the musculature of S. japonicum and that high potassium (60 mM) doesn't contract the muscle of S. japonicum, but the

potassium will depolarize the muscle (Bennett, unpublished information). Thus, sometimes the issues are not so clear-cut as one would like them to be when dealing with another parasite or even another species of the parasite. Also, it's interesting to note with respect to the response of S. japonicum to praziquantel that when we removed the tegument of the parasite we observed responses similar to those which we observed for S. mansoni; i.e., Mg^{++} would block the praziquantel-induced contraction. In another study we applied 100 μM of triton x-100 to a schistosome incubated in a balanced salt solution and observed that there was an immediate relaxation of the musculature, and the response of the musculature to an electrical stimulus became virtually nonexistent after about 10 minutes exposure to this detergent. Upon removal of the triton and replacement with Hank's balanced salt solution we observed that the parasite recovered and began to respond to the stimulus. It is interesting to note that while the triton is having this particular effect the parasite will also not contract in the presence of high potassium, but its musculature will contract when it is placed in praziquantel. When one combines these results (Depenbusch, unpublished data) with the fact that calcium movement into the parasite is severely retarded when triton is present, it would appear that triton may be uncoupling muscle contraction from the stimulus induced by electrical pulses or high potassium.

Lastly, with respect to the effects of drugs on musculature, it is interesting to note that the shistosome's longitudinal musculature (the musculature that runs along the long axis of the parasite) will respond to 5-HT with an increase in contraction rate but no change in tonus. When we measured the effects of 5-HT on circular muscle we observed a decrease in muscle tonus followed by an increase in muscle activity. This decrease in tonus doesn't occur in longitudinal muscle. It is also interesting to note that dopamine will cause the longitudinal muscle to relax, whereas application of dopamine to the circular muscle causes contraction (Pax, unpublished information). In addition, we have also examined the effects of drugs on parasite muscle which has been contracted by an electrical stimulus. This allows us to determine how a stimulus-induced contraction is modified by the presence of a drug. In the research that we have been conducting in this area we have found, for example, that if one stimulates the parasite and induces a contraction of the longitudinal muscle this contraction can be blocked by the cholinergic agonist carbachol and that this block is even greater when one uses an acetylcholine esterase inhibitor (Pax, unpublished information). All of this reinforces previous research indicating that acetylcholine plays a relatively significant role in inhibiting muscle contractility in S. mansoni. Equally interesting, if not more so, is the fact that after the longitudinal muscle of the schistosome has been stimulated we observe an immediate relaxation of the muscle, whereas with circular muscle a single stimulus to this

muscle results in the production of a contracted state; i.e., for 20 minutes following the stimulus the muscle is still contracted (Pax, unpublished results). All of this research on circular muscle and longitudinal muscle would indicate that there are significant differences, both physiologically and pharmacologically, between these two muscle groups. In fact it would indicate that the circular muscle is somewhat similar to the catch muscle that has been described by Tworg (1960) for clams.

With respect to our suction electrode and microelectrode work, we have developed a number of techniques to overcome the difficulties of working on small parasites. The suction electrode apparatus simply consists of one or more electrodes placed onto the surface of the worm and recording the electrical activity that is generated by the parasite. We are not penetrating the worm; we are simply picking up what electrical activity is generated from within the tissues of the parasite (Fetterer et al. 1977). We have observed that the electrical activity that comes from the parasite has the following characteristics. Lowering the calcium to .14 mM has a relatively dramatic effect on the high amplitude electrical activity being generated from the parasite. The low amplitude high frequency activity is not markedly affected. When the concentration of calcium is reduced to zero, one sees an effect not only on the high amplitude activity but a prominent effect also on the low amplitude activity of the parasite. Thus, it appears that the electrical activity being generated from the parasite is relatively calcium dependent (Semeyn et al., unpublished results). When we used high magnesium, which is often used to block synaptic activity, we observed considerable reduction in both low and high amplitude activity. Lastly, cobalt has a dramatic impact. Cobalt is known to be a very active calcium antagonist, and it markedly depresses electrical activity from the schistosome. When one measures electrical activity as a function of the various amplitudes and displays them as histograms one sees that the anterior, middle and caudal portions of the parasite have different frequencies of activities; i.e., from region to region these activities vary considerably. With respect to the effect of neurotransmitters on parasite electrical activity we have shown that carbachol (an acetylcholine agonist) will produce dramatic effects upon this activity in the concentration range of 10^{-4} to 10^{-8}. Even more interesting is a recent finding in which we observed that 5-hydroxytryptamine (10^{-5}) causes a considerable increase in the electrical activity of the parasite.

When we added a praziquantel isomer, which has no antischistosomal activity, at 10^{-10}M we saw no effect. When we placed the parasite in 10^{-5} 5-hydroxytryptamine in the presence of 10^{-10} praziquantel we observed a dramatic reduction in the electrical activity of the schistosome (Pax, unpublished information). Lastly, rather significant differences between

electrical activity can be observed in schistosomes that have an intact tegument in contrast to schistosomes from which the outer tegument has been removed. We observed that when 5-hydroxytryptamine is placed in the presence of the parasite the striking activity becomes much clearer, much sharper and much more prominent when the tegument of the parasite is removed.

In our lab we have also been involved in studying the various organ systems of the schistosomes, using techniques common to the field of electrophysiology. When one takes a fine tipped microelectrode and carefully manipulates the electrode deeper and deeper into the schistosome the following kind of events are seen on the oscilloscope. One can see an immediate drop in the polarity of the tip of the electrode from zero, which is the bathing solution, down to about -50 mvolts. Further advancement of the electrode leads to a movement of the potential towards nine to a voltage of about -32 to -27 mvolts, and then finally there seems to be a third compartment where the potential is between -8 to -12 mvolts. What are the areas within the parasite that are responsible for these particular potentials? We have answered this question by injecting horseradish peroxidase through the tip of the microelectrode and then removing the electrode and fixing the worm with gluteraldehyde. The parasite is then stained for the presence of horseradish peroxidase. When we place the electrode in the compartment which contains the -50 mvolt recording, we observed that the peroxidase ends up in the tegumental area (Fetterer et al. 1980, Bricker et al. 1982). When we stain for the area that maintains a potential of around -27, we see that the stain is in the muscle. These studies prompted us to use multiple electrodes. We are now placing two and three electrodes, separated by varying distances, in muscle and tegument to study the cable properties and the basic electrophysiological properties of muscles. Time does not permit me to describe this work, the results of which have been submitted for publication by Thompson and his colleagues.

Another area of work in electrophysiology involves the use of ion specific electrodes. We will be conducting research using electrodes that will detect the presence of specific ions within compartments of the parasite. Also we hope to begin to do more experimentation with an apparatus which we recently set up that will apply picoliter amounts of drugs to the surface and also to cells within the schistosome, using what we call a picospritzer. With this instrument, which will deliver picoliter amounts of a compound out of the tip of a microelectrode, we plan to measure the response of cells to the action of various drugs and thus circumvent the problem of putting the drug over the whole worm. This will allow us to characterize the receptive areas of the schistosome and determine exactly where these areas are located.

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DISCUSSION

BOTTJER: Your pictures clearly demonstrate there is acetylcholine and catecholamines in the schistosomes with which you are working. In some systems these comprise neurotransmitters that control the sympathetic and parasympathetic systems, one playing off the other. Would you discuss how you feel these two neurotransmitters may or may not play off one another in parasites?

BENNETT: As I said before, acetylcholine appears to be an agent which causes in general paralysis or slowing of muscle activity whereas 5-hydroxytryptophan does the opposite. It could very well be that these counteract one another in a way which would allow certain coordinated activity between the two opposing systems, such as relaxation of the muscle followed by contraction; and if this is rhythmic enough you could imagine that you could generate movement by having two opposing systems--relaxation followed by contraction. I think it is not well enough documented to show that there are opposing systems in Schistosoma. What we have studied is just the application of drugs from the outside of the system and a more refined method to really confirm whether or not there is this role of antagonism and agonism on certain muscle bundles. It's really yet to be proved, and has to be done at that level, before you can convince anybody of that type of interaction.

FRANDSEN: I was interested in your statement about results in regard to triton. I was wondering whether you have tried other non-ionic detergents or whether there is something peculiar about the triton?

BENNETT: Only triton. That's all we've tried. This is a compound that Cain and Oates used to elute off the tegument of Schistosoma. We kept playing around with different concentrations and all of a sudden we found that if you put 100 micromolars triton you not only block the stimulus-induced contraction, but you could throw the parasite into 16 millimolars potassium and not get a contraction. But as soon as you remove the triton you can get a contraction like that which occurs with high potassium and with stimulation. There seems to be a phenomenon operating that we know little about other than what appears to be a reduction in the rate at which calcium moves into the worm when you stimulate it. You get a depression of the calcium.

DESPOMMIER: There seems to be an interesting interface that hasn't been made yet, or at least appears to be possible, between the groups working on immunology of the Schistosoma and your group working on the electrophysiology of the organism. I was wondering if you thought about getting involved in that? I know it's a complicated area to begin with. There are many changes that the Schistosoma undergoes in its migration which

eventually make it non-susceptible to the host.

BENNETT: Right. I have a student who is now studying the membrane potential of eight-day-old schistosomula. Their tegumental membrane potential appears to be somewhere around minus 20 which is about 20 millivolts less negative than that of the adult. We are going to work with the younger schistosomula later on. Right now we have done about 10 or 15 experiments on the long stage, trying to gain a better understanding of its physiology. With respect to your question about our becoming involved in immunology, I might mention a recent experience of interest. Mike Phillips sent me some monoclonal antibodies directed against the tegument of Schistosoma. He sent them in a coded form, 1 through 15, and I did all of the experiments. There were three or four of the monoclonal antibodies that completely blew the tegument right apart; this was so obvious that one could almost detect it with the naked eye. Unfortunately, when I talked to him last week after returning from overseas, the individual who had access to the code was not available. Therefore, we still have to determine whether our method is worth anything with respect to analyzing the interaction of antibodies--at least monoclonal antibodies--directed against tegument.

DESPOMMIER: Have you ever tried to make direct physiological recordings from any of the neurological tracts?

BENNETT: Yes. We're in the process of trying to do that. Perhaps the best example is work employing the microelectrodes to which I referred in the presentation. With these we can inject into very specific locations picoliter amounts of a drug, enzyme, or other solution. With the technique, we hope to somehow get a better handle on some of the physiology and neurophysiology of the parasite. And also we're interested in using it to study the mode of action of praziquantel, using calcium specific electrodes (I should say "selective," for there is no such thing as a specific electrode) to measure calcium in the tegument before and after the addition of praziquantel.

DESPOMMIER: One final question, have you tried to measure the differences in any of those electrode recordings in the presence or absence of the opposite sex?

BENNETT: We haven't worked with differences between sexes except in the area of mechanical activity. We have considerable information on this but have not yet published it. We are interested in the possible influence of worms of the opposite sex on behavioral chemotactic factors. There have been some reports of influence of male on female or female on male behavior. We have never been able to find anything from the female that stimulated the male to become more or less aggressive.

CASTRO: You mentioned something about the contraction of the organism (male) around the female. It seems to me that contraction of the circular muscles of the male would cause the flaps of the gynecophoral canal to spread apart and pull away from the female rather than to contract around the female. I view the cross section of the male as being similar to a cross section of the gut. What makes it contract in the way that you describe?

BENNETT: There is an important point which must be taken into account relative to the data I have presented. We are working with a section of the worm. We have never taken a whole worm and turned it upside down and stimulated it to see if the entire musculature will completely collapse on a female worm placed on it. So what we're seeing here in the section may be totally different from what occurs in the intact worm. I understand the point you are making--that contraction should tend to pull the flaps up. But I don't know the exact geometry of the muscle system and how the oblique and longitudinal and circular are arranged. I only know the results of our studies.

CASTRO: How can you call the circular versus longitudinal?

BENNETT: It's really difficult to differentiate between longitudinal and circular. Our problem is conceptualizing basically what muscle alignment and insertion allows that kind of event to occur.

CASTRO: So it may not be just the circular muscles that are involved in holding the female. It could be diagonal or even longitudinal?

BENNETT: Right. It could be that they are selective muscles that are somehow pulling in ways in which, to us, just simply don't really make much sense.

THE SENSORY ABILITIES OF THE NEMATODE CAENORHABDITIS ELEGANS

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INTRODUCTION

An interesting and important question to ask about animals of all kinds is, "How do they perceive their environment?" What cues do they use to find food, mates, or shelter? Even bacteria make use of chemical gradients to acquire more favorable surroundings. Parasitic animals are particularly interesting from this point of view. They face the problem of locating a host which is evolving to avoid them. How do they do it? What cues are used? These questions are also of economic importance in that the answers might suggest new ways of disrupting the life cycle of parasites that damage human health, crops, or livestock.

Many parasitic animals are nematodes, and the behavior of this group is particularly interesting as they and their close relatives are the only animals with a cell-constant nervous system that is simple enough that the behavioral role of each individual neuron can be considered. Thus, studies of nematode behavior and neurobiology can be expected to provide information on the relationship of individual neurons to behavior with an unprecedented thoroughness.

The free-living nematode Caenorhabditis elegans has become an important model organism for studies in a variety of biological disciplines since it was selected by Brenner (1974) for its favorable genetic properties. In fact, C. elegans has become the most completely described multicellular animal in terms of cell lineages and neuroanatomy and competes with Drosophila sp. in completeness of the characterization of the genome. As a consequence, it makes good sense to concentrate initial behavioral studies on this species so that results can be related to the other types of information available on it, such as the wiring of the nervous system. Thus, C. elegans will be useful as a model for working out the general properties of nematodes, and this will provide a valuable basis for studies of specific

nematode parasites. For example, it has already been demonstrated that many neurons in C. elegans can be identified with corresponding neurons in Ascaris, the large nematode parasitic in animals (Ware et al. 1975, White et al. 1976, Stretton et al. 1978). In addition, upon starvation, C. elegans forms dauerlarvae which closely resemble the infective stage of many parasitic nematodes (Cassada and Russell 1975, Riddle 1978, Anderson 1978, Riddle et al. 1981). Thus, this review will concentrate on studies of this species. For a recent review of related topics see Dusenbery (1980d).

SENSORY ANATOMY

Most nematodes have six identical, symmetrically arranged sensilla that are close to the stoma. Each of these inner labial sensilla contains the endings of a few receptor cells (Coomans 1979). C. elegans appears to be typical in having two receptor cells per sensillum. The sensillum is constructed in such a way that one of these receptor cells is exposed to the exterior, while the other is embedded in the cuticle (Ward et al. 1975, Ware et al. 1975). The soybean cyst nematode, Heterodera glycines, has a similar arrangement, although it is a stylet-bearing species (Endo 1980). The anatomy suggests that one of the receptor cells mediates chemical stimuli and the other mechanical stimuli. In C. elegans the mechano-receptor makes a synapse on the most anterior set of medial muscles in the head. Thus, it may be hypothesized that this receptor is involved in controlling the position of the anterior tip of the worm.

Further from the stoma is another ring of six sensilla. These are generally referred to as the outer labial sensilla although differences have been detected between the lateral pair and the other four in several species (Coomans 1979), including C. elegans (Ware et al. 1975). Each of these sensilla also contains the endings of a few receptor cells (Coomans 1979). Again, C. elegans is typical, with one receptor ending per sensillum (Ward et al. 1975, Ware et al. 1975). In this case the ending is embedded in the cuticle with no opening to the exterior, so these sensilla may be presumed to be mechanosensors. In other species the presence of a pore through the cuticle suggests a chemosensory function.

A third ring of six sensilla is also found in nematodes. It consists of two distinct types. A cephalic sensillum is on each of the four submedial lips and an amphid on each of the two lateral lips. The cephalic sensilla are generally like those previously described. They contain the endings of only a few receptor cells and may or may not be exposed to the exterior (Coomans 1979). In C. elegans the cephalic sensilla contain a single neuron that is embedded in the cuticle and thus is presumed to be a mechanosensor.

The amphids are much more elaborate sensilla than those

previously described. In most species they consist of a duct which opens to the exterior and contains the endings of many receptor cells. The endings of other receptor neurons are associated with the sheath of the amphid (Coomans 1979). In C. elegans the endings of eight receptor cells are in the duct and appear to be exposed to the exterior, while four other neurons are also associated with the sensillum (Ward et al. 1975). Amphids have long been assumed to be chemosensory because of their anatomy, although little direct evidence exists. There is also evidence that in some cases they may have secretory functions as well. It has been suggested that, due to the probable role of the amphids of parasitic species in host finding, more attention should be given to their development in infective stages (Coomans 1979). In Neoaplectana carpocapsae it has been determined that the infective third stage has more elaborate amphids than the normal third stage (Poinar and Leutenegger 1968).

The phasmids are a lateral pair of sensilla on the tail of many but not all nematodes. They resemble amphids in general structure but are usually simpler. Each phasmid of C. elegans contains the endings of two receptor cells (Hall 1977). Again, the anatomy suggests a chemoreceptor function, but there is little if any evidence for chemoreception by the tail of any nematode except for the special case of males with a specialized bursa on the tail.

Many nematode species have a lateral pair of receptors called dereids back near the level of the nerve ring. In C. elegans each dereid has the ending of one receptor cell embedded in the cuticle. The ultrastructure closely resembles that of cephalic sensilla (Ward et al. 1975, Ware et al. 1975), and both types have the neurotransmitter dopamine (Sulston et al. 1975). The dereids are located at about the level where the head starts to taper, and it has been suggested that the function of the dereids is to signal that the animal has started into a passageway that is too narrow.

All the above sensilla have the same basic structure with two accessory cells. There are also several neurons that have endings in the head that are presumably sensory, although they do not have the organization of the sensilla (Ward et al. 1975, Ware et al. 1975).

A new type of nematode sensory cell has recently been discovered (Smith and Croll 1975, Chalfie and Thomson 1979). In C. elegans each of these cells is a neuron with a long, microtubule-filled process that extends along the body for a distance of about one-half body length. There are three pairs of such cells whose location is staggered such that nearly the whole length of the worm is occupied.

METHODS OF MEASURING SENSORY RESPONSES

A variety of methods has been used over the years to measure accumulation of nematodes in response to a stimulus. Recently, several new approaches have been described. The use of a slurry of Sephadex beads (Ward 1973) as a medium through which nematodes can move efficiently was a significant innovation. Although more expensive than agar, it has the advantages that it is chemically more inert, since it contains no charged groups, and worms can be more easily added or removed. The use of Sephadex has led to a very effective assay for attraction to chemical stimuli (Ward 1973). A small volume of the stimulus is placed at the center of a petri dish containing a Sephadex slurry. Appropriate time is allowed for the chemical to form a radial gradient by diffusion. The nematodes are added at the edge of the dish. C. elegans moves so rapidly through Sephadex that maximum accumulation is obtained in only 15 minutes.

Another method that appears promising for measuring attraction to chemicals is to place blocks of agar containing the attractant in a layer of water containing nematodes (Balan et al. 1976). The worms accumulate under the blocks containing attractant.

Countercurrent separation (Dusenbery 1973) is a radically different approach to the problem of measuring accumulation. It employs an apparatus consisting of an inclined tube in which a dense solution flows downward along the bottom, while a lighter solution, floating on the dense solution, flows upward along the top. After flowing through the apparatus the two solutions are collected in separate reservoirs. The density difference is obtained by adding sucrose to the dense solution. In order to increase the swimming efficiency of the nematodes, methylcellulose is added to increase the viscosity of both solutions about twentyfold. To test for accumulation, a stimulus chemical is added to one of the solutions, and the nematodes, suspended in an equal mixture of the two solutions, are injected into the center of the tube. After an hour or two most animals are found in the reservoirs, and the fraction that ends up in the reservoir with the stimulus is a measure of its response to it. With strong stimuli 99% of the animals respond. This method has the advantages that 1) large numbers of nematodes can be used, 2) the closed system permits use of volatile stimuli, 3) it works equally well for attractants and repellents, and 4) the stimulus concentration is within well-defined limits. The principal disadvantages are that the concentration gradient is complicated and the detailed behavior is not recorded. Thus, the method is generally not useful for studying the mechanisms of accumulation.

The analysis of the tracks made by nematodes is a very useful method of studying their behavior. Ward (1973) has improved the method by developing techniques for photographing tracks in

bare agar and establishing chemical gradients in the agar. Tracks often reveal much detail about behavior, and more studies such as those of Green (1966, 1977) would be valuable.

Frame-by-frame or slow motion analysis of movies of nematodes, or the video equivalent, is even more informative than tracks. Croll (1975) has demonstrated the usefulness of this approach. Although more complicated and time consuming than analyzing tracks, this method can record behavior not revealed by the tracks and provide precise information on the timing of various events.

A similar approach can be combined with a system to expose the nematode to chemical stimuli which can be controlled with a temporal resolution of a few seconds (Dusenbery 1980a). An individual nematode is held by the tail with a suction pipette. Its shadow is projected on an array of photodetectors which are connected to a multichannel recorder. This permits recording of the movements of the nematode with a temporal resolution of less than a second. As the behavior is recorded, solutions containing various stimuli are pumped past the nematode. As a result one can change from one well-defined chemical stimulus to another at a known and controllable time. This technique permits the study of many behavioral parameters such as adaptation that were nearly impossible to obtain with previous techniques.

SENSORY BEHAVIOR

C. elegans has been shown to respond to nearly a dozen different chemicals. The observations are summarized in table 1. These stimuli were identified by testing hundreds of common biochemicals (Ward 1973, Dusenbery, unpublished). All are attractive except for H^+, D-tryptophan, and CO_2 (or one of its hydrated forms) in phosphate buffer at pH 6.0, and high osmotic concentrations, which repel C. elegans. As far as has been determined, all these chemicals have thresholds within a factor of 10 of 10^{-4}M. It appears that few, if any, of these stimuli operate through common receptors. This conclusion is based on observations that cAMP, Na^+, Cl^-, and OH^- do not compete with one another (Ward 1973). And a set of mutant strains with various sensory defects indicates that responses to most of the stimuli can be independently eliminated (Dusenbery 1976b, 1980c).

A question of basic interest is how sensitivity to the various chemical stimuli is distributed among the receptor cells. This is a difficult question to answer in nematodes since electrical activity in these cells cannot be recorded.

However, two techniques are emerging which look promising. One is ablation of different receptors by genetic mutation. A number of mutant strains of C. elegans have been isolated that

Table 1.—Identified chemical stimuli for C. elegans

Chemical	Direction of response	Threshold concentration (mmol)	References
cAMP, cGMP	+	0.2	Ward 1973, Dusenbery 1976b
Na^+	+	2; 0.2	Ward 1973, Dusenbery 1974
Cl^-	+	2; 0.2	Ward 1973, Dusenbery 1974
OH^-	+	0.001; 0.01	Ward 1973, Dusenbery 1974
Pyridine	+	0.1	Dusenbery 1976a
O_2	+	0.2	Dusenbery 1980b
CO_2 (in borate, pH 8.8)	+	0.01	Dusenbery 1974
CO_2 (in phosphate, pH 6.0)	–	0.01	Dusenbery 1974
D-tryptophan	–	0.1	Dusenbery 1975
High osmotic pressure	–	150 (NaCl)	Ward 1973, Culotti and Russell 1978, Dusenbery (unpublished)

are defective in response to certain chemical stimuli (Dusenbery et al. 1975, Lewis and Hodgkin 1977, Culotti and Russell 1978). These strains are defective in response to various stimuli in a variety of combinations (Dusenbery 1976b, 1980c). The neuroanatomy of the head of the animal has been checked in several of these strains (Lewis and Hodgkin 1977). Contrary to some expectations, anatomical defects were found in about half of the strains, and different strains had different kinds of defects. Of particular interest was one strain that was defective in attraction to Na^+ and Cl^- and exhibited defects only in the inner labial receptor cell that is exposed to the exterior. All the other strains had defects in both inner labial sensilla and amphids and thus were not useful in indicating which kind of receptor cell was involved.

The second approach to this problem is the use of a laser microbeam to destroy identified structures (Sulston and White 1980). Initial experiments of this type indicate that destruction of the amphids (and probably other sensilla on the lateral lips) does not alter attraction to Na^+ or Cl^- but that destruction of all six inner labial sensilla (and probably other sensilla on the lips) does (Davis and Dusenbery, unpublished). Thus, some receptor on the lips other than the amphids is required. This experiment complements the genetic studies both in the sense that a different method is used and in the sense that a different type of sensillum is specifically altered. Taken together they provide strong evidence that the attraction to Na^+ and Cl^- is mediated by the inner labial sensilla. This is an important result, because attention has always been focused on the amphids as the site of chemoreception, and the

attraction to Na^+ and Cl^- is as strong and reliable as any other known chemically stimulated response in C. elegans.

Temperature, of course, has a general effect on most biological activities. In the present context we are interested in responses to temperature that suggest the existence of specialized thermoreceptors and adaptive behavioral responses to temperature. There are several (Croll 1970) reports indicating such responses in a few species of nematodes.

Ditylenchus dipsaci tends to accumulate at a certain position in a thermal gradient (Wallace 1961), and this eccritic (preferred) temperature is close to the temperature to which it has been acclimated (Croll 1967). Another series of experiments (El-Sherif and Mai 1969) indicates that this and another species have a remarkably acute sense of temperature. With electrical resistance and infrared beams, very small temperature differences were produced, and it was found that many of the nematodes accumulated at temperatures only 0.14 °C above ambient. A feature that should be pointed out is that this response is apparently quite variable in the sense that the nematodes may make it at one time but not at another. This is indicated by the fact that the initial response required half a day to become evident, while subsequent responses occurred in a quarter hour, and that only a proportion of the individuals made the response.

More recently it was demonstrated that C. elegans also has an acute thermal sense (Hedgecock and Russell 1975). It accumulates near its acclimation temperature. The eccritic temperature is reset in a few hours when exposed to a new temperature. Starved individuals and dauerlarvae appeared to disperse from the acclimation temperature. Of great interest is an observation that individuals located at the eccritic temperature in a thermal gradient often moved along isotherms for several centimeters, corresponding to 1-2 min and tens of body lengths, without diverging more than 1 mm, corresponding to 0.05 °C, from the isotherm.

Another interesting observation was that if individuals of C. elegans were placed in a thermal gradient many degrees from their acclimation temperature, they did not move toward it effectively and sometimes moved away from it. This type of behavior could explain the long delay before the nematodes responded to the small temperature differences in the experiments of El-Sherif and Mai (1969). If the nematodes were acclimated to a temperature (not reported) different from the ambient temperature of their experiments, the worms would not respond to the small temperature differences until they had acclimated to the ambient temperature. This would require several hours, as observed, if the rate of thermal acclimation is similar to that of C. elegans.

The above suggests that at least some nematodes have a very acute sense of temperature, although they may not always use it and it may be limited to a narrow range. A thermoreceptor has not been identified in nematodes, but the very detailed ultrastructural knowledge of the anterior of C. elegans (Ward et al. 1975, Ware et al. 1975) makes it a very promising subject. This is particularly true in view of the fact that single-gene mutations are known which alter the thermal response of C. elegans (Hedgecock and Russell 1975), and these might help identify the receptor.

Nematodes in general appear to respond to a variety of mechanical stimuli (Croll 1970). A particularly good series of experiments was carried out by Croll and Smith (1970). Their technique was to drop a pin through a tube from a known height, so that it would hit the nematode at a selected position along its length. A tap on the front half of a stationary Rhabditis sp. caused the animal to move backward, while stimulation of the posterior half caused forward movement. Casual observations indicate C. elegans also responds in this way. Repeated stimulation leads to adaptation. The lack of responsiveness lasted about 15 s. Interestingly, this is about the same time in which adaptation to chemical stimulation occurs (Dusenbery 1980a). Surprisingly, adaptation to anterior stimulation also prevented response to posterior stimulation.

More recently, Croll (1976) has used time-lapse video recording to analyze the behavior of C. elegans when it bumps into an obstacle. The usual response is to back up about half its length and then proceed forward in a new direction. Measurement of a variety of parameters indicated no correlation between the vigor of the response and the location or strength of the impact. The response thus appears to be a reflex that is simply triggered by the stimulus. In fact, the response resembles reversal bouts that C. elegans undergoes spontaneously (Croll 1975) or in response to chemical stimulation (Dusenbery 1980a). The surprising result is that impacts on the head where receptors identified as mechanoreceptors (Ward et al. 1975, Ware et al. 1975) are located do not produce detectably stronger responses. Croll concluded that these receptors do not mediate this response.

The explanation for this observation appears to be in the recently discovered microtubule cells (Chalfie and Thomson 1979, Chalfie and Sulston 1981). Stroking the front half of the animal with the tip of an eyebrow hair causes it to move backward, while stroking the rear half causes it to move forward. Elimination of these cells by lasar microbeam ablation or genetic mutation produces worms that no longer respond except at the very tip of the head. Furthermore, the two directions of response can be independently eliminated by ablating the anterior or posterior set of cells.

CONCLUSIONS

C. elegans appears to be endowed with a variety of receptor cells. In estimating the number of different kinds of receptor cells present, it is reasonable to assume that symmetrical sets of sensilla with similar anatomy mediate responses to the same kind of stimulus. Thus, for chemoreception one estimates eight kinds of receptor cells in amphids, one kind in the inner labial sensilla, and two kinds in the phasmids. This is about the same as the number of independent chemical stimuli known (Dusenbery 1980c). However, the simple hypothesis that each class of chemical stimulus is mediated by a different class of receptor cell is contradicted by recent results suggesting that both Na^+ and Cl^- are detected by the exposed neuron in inner labial sensilla. If more than one class of chemical stimulus can be mediated by each cell, then many more classes may remain to be discovered in C. elegans.

As mentioned previously, similar methods have been used to demonstrate that avoidance of a light touch throughout the length of the body is mediated by the microtubule cells. This leaves a variety of other receptor cells with undefined function. Most of these are presumptive mechanosensors. Some of them presumably mediate the touch sensitivity that remains at the tip of the head when the microtubule cells are eliminated. The cephalic and dereid neurons contain dopamine, which is presumably the neurotransmitter used by these cells to communicate with the rest of the nervous system. Mutations have been isolated that eliminate the dopamine and thus probably render the receptors inactive, but no effect on any sensory response has been discovered (Sulston et al. 1975). It would be interesting to know if these mutations eliminate the touch sensitivity remaining in the tip of the head after the microtubule cells are eliminated. Also, it must be remembered that somewhere in the worm is a thermoreceptor. Presumably, one of the types of receptor cell that is not exposed to the exterior is a thermoreceptor.

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DISCUSSION

FAYER: How do you differentiate sodium and chloride responses?

DUSENBERY: First of all, they're attracted to higher concentrations of sodium chloride, and the other part of the experiment is to give them a choice between sodium chloride and sodium sulfate. The concentrations are chosen so that the sodium is the same concentration in both solutions. And what we find is that they prefer the chloride over the sulfate. So they are responding to the negative ions in that case. And you can do the same thing with the positive ions. Give them a choice between the sodium chloride and calcium chloride, and they prefer the sodium chloride. So keep the counter ion the same in both solutions so they can't be responding to that, and the only stimulus has to do with one pair of ions.

FAYER: So you're always using the sodium chloride as one of your tests?

DUSENBERY: Well, we normally do. But you don't have to. I think we've done sodium sulfate and calcium sulfate or magnesium sulfate. We've tried a variety of counter ions. That does not seem to be an important part of it.

FRANDSEN: I was wondering if we know anything about concentration, for example of sodium, that the worm will respond to as compared to the internal concentration? This is obviously connected with the question, "Does it work to maintain its isotonic condition and seek an environment where the amount of work expended to maintain itself is minimal?"

DUSENBERY: Yes, I left out a relevant slide. We've asked the question, "What is the preferred sodium chloride concentration?" We've done that by giving the worm a choice between one concentration and half that concentration, and testing a variety of such pairs. If you do that, you find they are attracted to higher and higher concentrations up to 100 millimolar. If you go above 100 millimolar, they're attracted to lower concentrations. So the preferred concentration is just about 100 millimolar; presumably that's isotonic. You don't know exactly what the concentration is inside, but that certainly is the physiological range. So I think that's exactly right. They prefer the concentration that's isotonic.

BONE: At that point, David, do you find arrhythmic waves? What happens to the body waves as far as reversal of the optimum?

DUSENBERY: Well, the behavior looks just the same. It looks just fine at 100 millimolar sodium chloride.

BONE: You just said they were going up and then down. Do they not "freeze" at some point? I'm thinking of the population in which you can typically see a plateau at a certain level of stimulation. What is happening at that point?

DUSENBERY: Those experiments were not done with a gradient over the whole range but with the tethered worm assay. We're just giving them a choice between two concentrations at one time. Presumably if you give them a gradient and start them low, they will go up until they reach 100 millimolar and stay at the concentration.

BONE: There are no body waves at that point?

DUSENBERY: Well, they move around. In fact, the normal culture medium is about 50 millimolar sodium chloride. Presumably they wouldn't go up and down the gradient. They would move back and forth across it.

CURRENT: These worms are only a millimeter long. If you're using anterior to posterior differences to detect 10^{-4} molar, do you think that would really work in nature to see that much of a difference over that small a distance?

DUSENBERY: Well, the natural concentration would be higher than that. Typical concentrations in soil are more like millimolar. But aside from that, the question is still a good one. I don't know; I think it's very complicated. You would have to worry about how much time they could integrate sensation over before making a choice about the stimulation. I don't see any simple way to answer that.

WILT: I am a bacteriologist; this helps explain my lack of knowledge about worms. Several years ago we studied the interaction between bacteria and nematodes. We have assayed five types. Two of them had been identified by Rodriguez-Kabana, plant nematologist here at Auburn. One was a species of Caenorhabditis; the other was Peloderum chitwoodii. Most of the work that has been published was on that particular one. We were interested in the kind of bacteria that these free-living nematodes feed on. It is commonly stated that they feed on detritus. We isolated these worms from the natural habitat, washed them, and ran plate counts on the washings so we would know if we had the nematodes washed free of bacteria. Then we crushed them in a tissue grinder and invariably the count would go way up once the bacteria were released, presumably from the gut of the nematode. Later, one organism, Nectrin vialocium, was isolated that was very toxic to the nematode. It produces a nonwater-soluble purple pigment. We got some rather interesting results. Most of the nematodes moved to the yellow bacteria. However, we knew that some of them had fed at the purple cells. So that led to some migration studies in which a number of variables were used, including CO_2, live versus dead bacteria, sterile versus nonsterile soil, and concentration of bacteria. It was very clear that some of these nematodes were attracted directly to bacteria. We recorded some of the migration patterns on film, using time-lapse photomicrography.

DUSENBERY: Thank you. I'm convinced that there are a lot of attractants that bacteria release that haven't been identified.

WILT: One further comment. We did find certain amino acids that were attractants. But as I listen to what you're saying here, I can think of many things I didn't know back in those days. In the environment in which these free-living nematodes are found, bacteria are not scattered evenly in the soil, nor are they evenly distributed along intestinal mucosa. Rather you have microhabitats, in some of which there is an increase in microbial activity. These environments very likely are not anaerobic; they are certainly microaerophilic in many cases. This tends to favor reduction of organic acids, alcohols, etc. Also, are there within bacterial cell walls lipopolysaccharides

or other polysaccharides that tend to be attractive to bacteria-feeding nematodes? Finally, it's very interesting that you say that tryptophan is repellent, because E. coli metabolizes tryptophan to indole. And the only difference in the molecule is that you lose the side chain. Would indole also be a repellent?

DUSENBERY: Actually, I've looked at indole. It's not a good stimulant either way.

DESPOMMIER: I was going to ask whether or not anybody has ever pulled apart the medium of E. coli and done some chromotography testing for an attractant?

DUSENBERY: I have not done that. I don't know that anyone else has. It's likely some people in some of the labs have had some students play around with that. There is nothing that has been published.

DESPOMMIER: There are many, many more mutants in E. coli than there are of Caenorhabditis. Is it possible to explore its food-seeking activities by just looking at different mutants of E. coli?

DUSENBERRY: It's certainly possible. Well, in fact, Sam Ward used cyclic AMP mutants to see if that was the stimulus involved in the attraction of E. coli. And the mutants lacking cyclic AMP were still very attractive to the worm. So cyclic AMP clearly is not the whole story for attraction to E. coli. The mutants are very useful to make a nice, really clear-cut experiment. But I think that just fishing through mutants in general hoping to stumble on one that's not attractive might be a long search.

DEVINE: I have a couple of questions. First of all, do they show any photo response?

DUSENBERY: Some people, including myself, have played around with that. I don't believe they do. But they have a very exquisite thermoresponse, and it's difficult to really be sure that you are eliminating all temperature effects from the light stimulus. People frequently observe a response by shining a bright light on the worm. And they will avoid the light. But I think it's probably a thermoresponse.

DEVINE: The other question is, if they are indeed orientated by the comparison of stimuli between head and tail, how would you carry your hypothesis over into asphasmic nematodes?

DUSENBERY: Well, I wouldn't carry it over.

DEVINE: Would you care to speculate on what you think is going on in those systems?

DUSENBERY: Well, there's no reason you can't find your way up and down the gradient with just sensors on the head. After all, E. coli can work its way up and down the gradient with a single cell being stimulated. It doesn't detect any spatial gradient. It's all a matter of temporal changes of stimulation as it moves. The nematode can do that perfectly well, too, with stimuli only working on the head. If things are getting better, it keeps on moving in the same direction. And it can work its way up and down the gradient that way. There's no real necessity for head and tail stimulation or head and tail comparison. In my mind it's a very interesting question though, because for a wormlike animal it's a highly rational way to design a detection system. It could be much more sensitive, because you can compare stimuli over a fairly long distance over which to place two receptors. But a wormlike animal does have that opportunity. And the surprising thing to me is that I don't know of any cases that have been demonstrated for any kind of wormlike animal where it's clearly been established that the worm compares head and tail stimulation to work its way up a gradient. There are some suggestive studies, but I have not seen any that are really solid.

LONG: Can I ask a rather simple question? This movement of the nematode, all the movement is done by being on a solid surface, isn't it? It cannot move freely in liquid solution?

DUSENBERY: Caenorhabditis does not move well in deep water.

LONG: The general way, though, is they can't move in a solution. They have to be on a solid surface to move. How does that work out in the counter current?

DUSENBERY: Well, the worm swims basically back and forth between the top and bottom of the tube in order to choose which solution it's in. Most of its movement along the length of the tube is due to a flow of the solutions. But the worms can swim. And we have helped them by adding methylcellulose to the solution to increase the viscosity about twentyfold. They certainly don't move as efficiently as in a film of water. But they can swim a little bit that way.

ROBERTS: Dave, we've got you back-pedaling a little bit already on your spatial-detection hypotheses. I'm going to keep you back-pedaling a little bit and ask you another question, and make a comment. The question is, "When you mutate taxis to sodium chloride, does that then lead to repulsion by sodium chloride, which essentially is the genetic corollary of the laser ablation experiment?"

DUSENBERY: That's a very good question. And I have two answers for it. One is that some of the mutants do have reverse responses to sodium chloride. The other is that no one has looked at the anatomy of the phasmids to see if they are disrupted or

not. Lewis and Hodgkin, who worked in Brenner's group, have isolated some chemotaxis mutants. But instead of studying the behavior in detail the way I did, they did electron microscopy of the mutants to see that sensilla were altered. And the general findings were that in most of the mutants all of the sensilla in the head were altered. I would bet the phasmids are altered, too. They didn't look at the tail. So those mutants wouldn't be relevant to your question. They did have one mutant strain which really supports our observation with the laser. And that was a mutant strain which appeared to have normal amphids but an alteration in the inner labial sensilla. And it was not attracted to sodium or chloride. So that's a genetic ablation that fits in our laser results. There is a problem on that point, however, because Don Riddle's group has just published a paper on mutants altered in dauerlarvae formation in Caenorhabditis. It turns out many of these mutants are defective in chemotaxis. They have done electronmicroscopy on many of their mutants. And they describe one in which they saw alteration of the amphids, but not the inner labial sensilla. And that mutant was defective in sodium chloride. So we have really got a contradiction now between those two different genetic strains. And one can, unfortunately, always discard the electronmicroscopy by saying, well, there's a defect that he couldn't see by electronmicroscopy. I think that is the reason that Lewis and Hodgkin did not draw the conclusion from their original observation that it wasn't the phasmids because of that weakness of the argument. They just described the mutants and didn't go on to draw the conclusion that the amphids were not the site of detection of sodium chloride. Thus, I think the laser experiments really are somewhat more clear-cut.

LONG: Let me add a comment, and I would like to hear your response. My comment is that if an animal is going to spatially detect a gradient, it should be simultaneously comparing concentration at two different locations. If you knock out the anterior detector, the posterior detector--in this case the phasmid--has nothing to compare concentration with. I would think that that would tend to confuse the animal more than help it, or that you would have to separate temporal detectors working in some sort of coordinated mechanism.

DUSENBERY: I would agree. You don't know what's going to happen when you damage the amphids. You don't know what the sensation is going to be of having your receptors destroyed. I'm guessing it's the sensation that's equivalent to no stimulation. But that's a guess.

CHEMOTAXIS OF PARASITIC NEMATODES

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Our understanding of the chemosensory behavior of nematodes has undergone considerable expansion in recent years. However, the vast majority of the research findings have concerned the chemical orientation of strains and mutants of the free-living nematode Caenorhabditis elegans, as recently reviewed by Ward (1978) and Dusenbery (1980). Examination of the above reports gives significant insight into nematode chemoreceptors, their sensitivity and specificity, and the resultant behavioral neurology of nematodes but also allows evaluation of our remaining informational gaps.

Regrettably, if one seeks to find similar knowledge of the chemically-mediated behavior of parasitic nematodes, the paucity of the literature is evident, despite the apparent importance of chemical stimuli to a number of plant and animal parasites. However, the recent summaries of nematode sense organs (Wright 1980) and Ascaris locomotion (Johnson and Stretton 1980) establish an excellent background for a rapid growth in our comprehension of chemosensory phenomena in parasitic nematodes to rival that of the more widely investigated free-living forms.

Nematode behavior may not always be the result of chemotactic orientation or chemically-mediated selection of habitat but in many cases should probably be considered as a localized event within an organ or tissue which was reached passively. Wilson (1977) has supported the concept that Strongyloides ratti exhibits larval accumulations that result from the structure of the pulmonary vasculature rather than any behavioral or chemosensory response while Hawking (1975) has stated that the accumulation of some microfilariae is a departure from a random distribution due to oxygen tension. This elevated oxygen tension in the pulmonary arterioles causes the larvae to exhibit body waves that are directed anteriorly or "go into reverse" and actively remain in a passively-acquired habitat.

Larval behavior of Nippostrongylus brasiliensis is also considered as a non-oriented phenomenon by Croll (1977a) and Croll and Ma (1978). Larvae are passively trapped in the pulmonary capillaries. Emergence from this organ is a nondirectional event which may be accomplished by feeding activity, presumably as a result of unknown gustatory stimuli.

Croll and his coworkers (Croll 1976, Croll and Ma 1977, Croll and Smith 1977, and Croll et al. 1980) have also redefined the orientation behavior of larval and adult N. brasiliensis in the rat host. Adult worms in the intestine showed considerable tolerance to intestinal conditions with their distribution being determined primarily by the pattern of food. Nematodes in the food bolus became inactive until the quality (or perhaps quantity for the population) became inappropriate. However, a host secretion or food by-product was also implicated as an attractant or repellent for the longitudinal chemotaxis of adults within the intestine.

Although many events of localization in the nematode's existence are regulated by host anatomy, general agreement is found in the literature regarding the importance of chemosensory messages to most stages of the worm's life cycle. Thus, a number of studies have concerned the orientation of zoo- and phytoparasitic larvae toward their respective hosts. Review of these earlier reports will not be attempted, since the summaries of Croll (1970, 1972, 1975a) and Green (1971) present much of the literature.

More recently, further work on chemotaxis by parasitic larvae has become available. Pye and Burman (1981) found that larvae of the insect parasite Neoaplectana carpocapsae accumulated at cationic sources of Na^{+}, Cs^{+}, Mg^{+2}, and Ca^{+2} in order of decreasing thresholds for maximal response. Potassium acetate did not elicit a response by the larvae, while NH_4^{+} apparently had repellent activity as did high PO_4^{-3} levels. The anions CO_3^{-2} and Cl^{-} also attracted N. carpocapsae larvae, but SO_4^{-2} revealed no effect.

N. carpocapsae larvae also moved toward pH's over 8.6 and were repelled by pH 2.5. Random distributions were found from pH 3.8 to 7.6. Gram negative bacteria and heat-killed Pseudomonas aeroginosa were attractive to the larvae, but no response was found with a chemical source from gram-positive bacteria. In addition to chemotaxis to bacteria, a number of fungi may release attractant or repellent chemical cues for nematodes (Jansson and Nordbring-Hertz 1979).

The nucleotide c-AMP causes accumulation of N. carpocapsae larvae. However, the sugars glucose and trehalose and the amino acids histidine, glycine, and proline elicited no response up to 37 millimole concentrations. Pye and Burman (1981) proposed that at least three response systems (CO_3^{-2}, bacteria, and

other ions) were present, based on potentiation and inhibition and inhibition studies with mixed chemical sources. Croll (1977b) has summarized information that indicates an extensive chemoreceptive discrimination, at least in wild C. elegans. Chemoreception of a wide variety of compounds is probably generalized throughout the nematodes.

Somewhat in contrast, larvae of Trichinella spiralis were repulsed by a KCl gradient, but activated in a non-directional manner by lactic acid (Hughes and Harley 1977). Glycogen and phosphocreatine caused no response by T. spiralis. Stringfellow (1981) examined the responses of sheathed and exsheathed larvae of Ostertagia ostertagi to various host components. Neither larval stage responded to Na^+, although the pH created by 0.1 M $NaHCO_3$ was attractive. The chloride ion, which is present in the gastric contents, was suggested to have repellent properties.

The kinetic activity of Dictyocaulus viviparus in response to various chemicals was tested by Jorgensen (1980). However, the experimental protocol did not allow determination of taxic orientation to a source but only population activity levels. Bile increased the motility of infective larvae but depressed movement of pre-infective nematodes. Differential effects were found with various bile salts, although detergents gave no significant responses. Pepsin, trypsin, and high CO_2 also stimulated larval migration. Phocanema decipiens larvae also become active in pepsin and HCl at pH 1.6 (Croll et al. 1980). Any orientation to these digestive secretions remains unknown, although orthokinesis could lead to the accumulation of larvae at a chemical source if a gradient is established and the larvae remain responsive after initial activation.

As indicated above, there are discrepancies in the larval responses of different species to the same chemical stimulus. These differences may result from the biological or ecological relevance of the message to the individual larvae or may actually result from the usage of different bioassay systems and/or testing of limited or even single dosages.

Our awareness of chemotactic responses by adult parasitic nematodes is less than that of the larval stage. As indicated by the following discussion, the list of known compounds that elicit responses is not extensive, and their behavioral purpose is often obscure. However, a variety of intriguing leads can be and undoubtedly will be drawn from investigations of the free-living representatives, such as C. elegans.

Chemical factors from the host probably exert considerable influence on the chemotaxis of adult parasitic nematodes. However, there is little direct evidence to discuss. Cunningham (1956) found that the lung-through-adult stages of N. brasiliensis would respond to host serum, although no constituent

compounds were identified. Additional study of feeding stimulants, using known compounds, may prove enlightening.

Adults of N. brasiliensis will respond to cyclic nucleotides (Nordstrom and Bone, unpublished). Males were significantly attracted to 1-5 millimole concentrations of 3'-5' c-AMP, but higher concentrations caused movement away from the source. Other nucleotides such as 2'-3' c-AMP, c-UMP, c-IMP, and c-CMP had no effect, while c-GMP and c-TMP elicited repulsion of the worms over a 1000-fold range. The negative response to c-GMP by male N. brasiliensis differs from that found in the free-living nematodes. Willett (1980) has reviewed the occurrence of c-AMP in Panagrellus redivivus and C. elegans and of c-GMP in P. redivivus. Our unpublished studies have readily found c-AMP in extracts of female N. brasiliensis by ion exchange chromatography, but no c-GMP has been detected. Further study of the occurrence of cyclic nucleotides in parasitic worms and their chemotaxes could prove interesting.

No information is apparent regarding the orientation of nematodes to biological amines, although Croll (1975b) has reported that nematode muscular contractions are caused by serotonin, 5-hydroxytryptophan and epinephrine. Mettrick and Cho (1981) found that the tapeworm Hymenolepis diminuta moves anteriorly in the intestine toward a serotonin source in a dosage-dependent manner. Kelly et al. (1974) stated that intraduodenal injections of histamine or serotonin had no influence on expulsion of N. brasiliensis, although some prostaglandins reduced (repulsed?) the worm burdens significantly. However, the protocol was not designed to determine any chemotactic responses by the helminths. Examination of the orientation responses of nematodes to the biogenic amines should be conducted, based on the above background.

In contrast to the limited information on responses to known compounds, our laboratory has investigated various parameters that influence pheromone chemotaxis. However, the chemical compounds that are responsible are unknown, even though certain chemical features are understood.

Pheromones are chemicals that are released to the exterior of an organism and that elicit some behavioral or physiological response. With the recognition that pheromone disruption or confusion represents a biorational means of controlling injurious invertebrates, a vast expansion of our knowledge of insect chemocommunication has occurred in the past decade (Shorey and McKelvey 1977). Intuitively, it appears reasonable that if insect management can be partially or totally achieved by manipulation of chemical messages, similar schemes for nematodes are feasible.

A summary of those parasitic nematodes and facultative parasites which have been studied for pheromone communication is

given in table 1. As shown by the limited information, our knowledge is confined to eight parasitic genera. Moreover, most of these reports represent only solitary investigations with the exceptions of Heterodera, T. spiralis and N. brasiliensis. A more complete listing and recent review of pheromone-mediated nematode behavior can be found in Bone and Shorey (1978).

Chemotaxis by male nematodes to female pheromone is influenced by a diversity of factors. The ability of sexually-mature male N. brasiliensis to respond to female pheromone declines with increasing age. This decrease is considered to result from sexual senility and also host-immune damage. The loss of responsiveness with aged males was not as great in vivo as in vitro, which suggests that artificial bioassay systems may have a minor influence. The onset of male reception of female pheromone is apparently coupled to the final larval molt so that males become responsive at sexual maturation by in vitro bioassay. Again, in vivo studies show enhanced male responsiveness in younger males which do not respond significantly in vitro.

Although pheromone chemotaxis is age- and immune-dependent, no influence has been found for time-of-day or mated versus virgin males. Males can theoretically inseminate up to 4.6 females per hour; thus they apparently remain responsive to pheromone

Table 1.--Pheromone communication in parasitic nematodes

Species	Sexes attracted	References
Ancylostoma caninum	M	Green and Plumb 1970
Aspiculuris tetraptera	M	Roche 1966
Cammallanus sp.	M,F	Salm and Fried 1973
Heterodera rostochiensis	M	Green 1966
Heterodera schachtii	M	Green 1966
Heterodera avenae	M	Green and Plumb 1970
Heterodera carotae	M	Green and Plumb 1970
Heterodera cruciferae	M	Green and Plumb 1970
Heterodera glycines	M	Green and Plumb 1970
Heterodera goettingiana	M	Green and Plumb 1970
Heterodera mexicana	M	Green and Plumb 1970
Heterodera tabacum	M	Green and Plumb 1970
Heterodera trifolii	M	Green and Plumb 1970
Nippostrongylus brasiliensis	M,F	Alphey 1971
Pelodera strongyloides	M,F	Stringfellow 1974
Rhabditis pellio	M,F	Somers et al. 1977
Trichinella spiralis	M,F	Bonner and Etges 1967

until metabolically-exhausted. Actidione, an inhibitor of protein synthesis, does not block chemotaxis by males to females, but treatment of males with synthetic insect juvenile hormone as larvae will reduce their in vitro responsiveness.

Physical parameters, such as those established by in vitro bioassay designs, will also influence the chemotaxis of male N. brasiliensis. Chemical gradient formation is time-dependent so that simultaneous placement of responding males and female pheromone in the bioassay devices reveals little male movement until after a one-hour diffusion period. Introduction of males into a three-hour gradient also reduces the male's orientation when compared to the results from exposure to a two-hour gradient. Thus, gradient formation should be a prerequisite study in the physical dimension of any in vitro bioassay device. The diffusion of a chemical gradient is also distance-dependent. In vivo bioassay of posteriorly-placed males to anterior females after surgical transfer into the mouse intestine showed a decline in male movement as the distance between the sexes was increased. Anterior males failed to orient to posterior females in the intestine, a finding which suggests that peristaltic flow may partially control mate location in the intestinal environment.

Several biological parameters exert various effects on male chemotaxis in Nippostrongylus. Sensory adaptation and/or habituation of the male's movement can be produced by preexposure to the female compounds prior to testing of the male's response to living females. Tactile stimulation among groups of males will also reduce or inhibit their collective response to female chemicals in T. spiralis, Camallanus, and Nippostrongylus (Bonner and Etges 1967, Alphey 1971, Salm and Fried 1973). Bone and Shorey (1977) and Bone et al. (1978) found that male-to-male chemocommunication would also decrease responsiveness to female pheromone when the male interaction occurred prior to or during exposure to a pheromone gradient.

Chemotaxis of males is increased when a chemical source from mixed-sexes is employed. Although part of this phenomenon is probably a result of increased production of pheromone by females, some experimental evidence suggests that more than a single compound is involved so that multiple chemoreceptors of the responding males may contribute to the enhanced locomotion.

Chemotaxis of adult female Nippostrongylus remains largely unknown due to our emphasis on the females as a chemical source. However, the females' response to conspecific males is not dosage-dependent but is significant at most tested dosages. This is in direct oppositon to that found for the male worm. Our unpublished research failed to demonstrate any in vivo attraction of posterior females to anterior males within distances at which the males were found highly responsive. The scant information on chemotaxis of females to known compounds

or pheromone precludes any conclusions at this time other than to reiterate that basic chemoreceptive or behavioral differences probably exist between the sexes but remain largely unexplored.

Hybridization between nematodes has been shown for a number of genera, such as Ancylostoma, Haemonchus, Brugia, Angiostrongylus, and Cooperia (Rep et al. 1968, LeJambre 1979, Bhaibulaya 1974, Isenstein 1971, Suswillo et al. 1978, Setasuban 1977). Generally, these studies have concluded that copulation and mating may occur but that the viability of the hybrid offspring may be altered detrimentally. Thus, chemotaxis of nematodes to the opposite sex probably takes place in the host due to the presence of similar functional groups on the inter-specific pheromones and mutually-sensitive chemical receptors.

Several in vitro studies have been conducted on the specificity of nematode pheromones. Roberts and Thorson (1977) reported interspecific pairing between N. brasiliensis, Nematospiroides dubius and T. spiralis. Green and Plumb (1970) examined interspecific attraction in Heterodera. More recently, Belosevic and Dick (1980) have examined three Trichinella isolates and reported differential patterns of chemical attraction. However, from the above studies, only Trichinella was tested in a dosage-response format which is necessary to detect subtle differences.

Based on our limited knowledge, one may propose that many nematode species may not be completely isolated by pheromone-mediated chemotaxis, although levels of specificity in chemical production and/or chemical reception probably do occur. Other mechanisms, such as developmental and ecological factors, may actually create reproductive isolation rather than chemical specificities. Such a situation is found in two noctuid moths which utilize the same pheromone but differ greatly in chemical production and reception threshold (Kaae et al. 1973).

Our pheromone studies seemingly indicate that certain methodological approaches should be used for study of nematode chemotaxis. The sensory adaptation and diffusion time data suggest that all studies should employ dosage-response analysis across a range of concentrations. Extrapolation from a single dosage fails to reveal any significant increase or decrease in chemotactic behavior which occurs with various biological and environmental changes. Nematode repellency can be produced readily by over-saturation of in vitro bioassay devices. Thus, compounds such as c-AMP or female pheromone that cause positive chemotaxis can easily be interpreted as repellents with the improper selection of a high concentration. Dosage-response analysis across a 10^3 or 10^4 range would appear mandatory for reproducible advancements of our knowledge.

Equally as important, standardization of bioassay techniques or

devices within biological constraints would offer comparableness between research groups and organisms. The use of gel or agar plates for larvae and free-living nematodes may offer partial resolution of this need. However, no apparent trend is evident for future research on chemotaxis by adult zoo- or phytoparasitic nematodes.

Obviously, additional research should be performed on other species--in particular, the agriculturally and medically important parasites. However, the laborious and expensive adherence to a dosage-response format will probably retard many advances until considerably more information becomes available from model systems. Once the logistics and chemistry are developed, one can readily foresee the implementation of pheromone-based control schemes for a number of devastating nematodes.

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DISCUSSION

FRANDSEN: What is the molecular weight range?

BONE: One is slightly under 400. The other is approaching 600, which suggests five amino acids if you take 120 as the standard residue weight.

CASTRO: So you have two proposed attractants?

BONE: We have three in the total communicative system. In the male we have one which has a similar weight to that in the female but a different isoelectric point apparently, based on its absence at a 7.3 pI.

CASTRO: Those would both be peptides?

BONE: Those would both be presumed peptides. In the female we have the lighter weight, organic-soluble component, which we don't find at that elution region in the male.

CASTRO: Do you have any speculation on what structure that is?

BONE: The lighter weight one? I could go as far as to say it's apparently a hydrocarbon, based on its solubility.

CASTRO: Which leads me to ask you to speculate on hormones in the nematodes. And how they may play a part.

BONE: They are present, but their involvement is mostly unknown, based on very scant literature. We haven't really put the two together but have started work in the area. Reading between the lines, in the crayfish we find that ecdysone is released when the final molt takes place, and then ecdysone acts as a pheromone to attract the male. At the proper time of the sexual receptivity, no additional bioenergetics are required to do so. We have considered the dual role of a hormone as a pheromone but really don't have any information at this point.

BENNETT: I know there's a considerable number of compounds that can inhibit pheromone synthesis quite specifically and at extremely low concentration. One, have you ever tried to use these compounds and, number two, just as a technical comment on your separation of your protein from your low molecular weight organic, I've had a similar problem in another context and have found that using a rather complex computer-driven tertiary system gives a nice clear separation.

BONE: Can I give you an easy solution perhaps that may work for you? We've also used reverse-phase-sep-pacs, and with a methanol series we can hang up the pheromone fraction. In fact, we actually use it as an enrichment procedure. The maximal retention on a C-18 sep pac is about 20,000 female

hours. After that you get a linear decrease, which I presume is due to the fact that the matrix is saturated and everything else is going through. The problem is that we never have had enough worm-hours to fill up all the available sep pacs.

BENNETT: And the inhibitors?

BONE: We haven't gone into that in any detail. Actidone, a protein-inhibitor, will block female production. Most recently we have used several synthetic juvenile hormones that are active in insects and can block pheromone production by treatment of the female nematode. There are effects of juvenile hormones on nematode reproductive systems.

DESPOMMIER: Have you ever tried to inhibit in vivo the reproduction?

BONE: Yes; I should have mentioned that. If we pre-expose male to female incubate, give them enough sensory insult, they won't respond to the anterior females in the gut. Eventually, we hope to develop a microencapsulation technique so the gut could be permeated with pheromones and communication fails.

WILT: You're talking about a peptide that's only four or five amino acids in length. It would seem to me that bacteria would gobble that thing up as fast as it could be produced by the female. If I'm not wrong, is this your suggestion that this is produced in the small intestine?

BONE: Yes, I think we perhaps have a counter balance. The problem in pheromone communication is that first of all one must deliver a message, but one must also prevent formation of false messages. So it's only logical to assume that the pheromone has limited stability and a natural breakdown, or is swept away by peristaltic flow. Therefore, the false stimuli in the environment are eradicated and the location of a natural source is enabled. We have examined the effects of bacteria on the pheromone solution and found no significant increase in degradation.

WILT: Then do you think there's any possibility that you may be looking at a determinate group on a larger protein?

BONE: That has been suggested before, but I really can't provide an answer at this time.

WILT: Solubility may be another key.

BONE: Solubility is a factor in some initial work we have done with Heterodera, the cyst-nematode. With adjustment of the pH to certain regions, which are outside of the typical Illinois clay, there's no attraction of the male to the female. One of the most immediate answers is that at a certain pH the presumed

protein may precipitate out at its isoelectric point and not move, if Heterodera has a protein as we feel Nippostrongylus does.

DESPOMMIER: Have you exploited the specificity of this pheromone with regard to the living nematodes to see whether or not it can attract others?

BONE: No, we haven't, but Dr. Roberts has found some cross-attraction, although the pattern differed. Dr. T. A. Dick has also looked at Trichinella and found some cross-attraction, but in a different pattern. In the literature on nematode reproduction, there are a number of cases of hybridization between various nematodes, which suggests that there is some "cross talk" between the species. If so, then you can apply the same pheromone compound to multispecies rather than a single species for biocontrol.

DESPOMMIER: Have you investigated receptors then?

BONE: No. And based on the other presentations, anything I would say would be superficial and very naive.

DAMIAN: I believe you mentioned that pheromone production was reduced in the older females?

BONE: Yes.

DAMIAN: You attributed that to either immune damage or perhaps to built-in senility. I just wanted to mention that Ogilvie in her earliest experiment with Nippostrongylus was interested in that possibility with respect to their expulsion. She did some serial transfers in naive rats and found no evidence of senility in worms. Also, have you done any of the transfer experiments in the immune rats?

BONE: You mean to avoid the immune reaction?

DAMIAN: No, just to see if there's a difference in attractiveness to the immune environment as opposed to a naive in vivo.

BONE: Dr. Roberts has shown that in immune response, at least at that immune period, pheromone communication is damaged. Our in vivo work has also shown immune damage on sexual communication. In response to your first statement, worms in immune-suppressed hosts show decreased egg production, which I view as senility.

FRANDSEN: Has there been any attempt made to ascertain any effectiveness in the nematode system of proven pheromone from insect sources?

BONE: No. It's a good suggestion, and there are hundreds of compounds to work with.

BENNETT: Where are the receptive fields within either insects or parasite nematodes to the pheromones?

BONE: In insects they are commonly in the antenna, and chemosensory stimuli can be measured by electroantennographs or EAG's.

BENNETT: In the nematode? Do you have any idea?

BONE: There is no general agreement in the literature, and we haven't really explored the mechanism. Much of our work has been aimed toward the chemical isolation and identification, and with that ultimate goal, we have neglected many other areas such as receptor physiology.

BEHAVIORAL CUES IN TREMATODE LIFE CYCLES

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INTRODUCTION

Understanding of chemotaxis in trematodes has lagged behind that of certain of the other parasite systems. This may be due in part to the fragility and ephemerality of extra-host stages of the most studied trematodes. One great advantage to choosing trematode systems for such investigations is the variety of different behavioral adaptations of trematodes to host-finding activities. This paper is limited primarily to the schistosome model system for two reasons: (1) the general biology of that trematode has been best studied and (2) this model system may help to understand chemotaxis in trematodes which have a more complex life cycle.

MIRACIDIA

Miracidial behavior and host-finding is a well-reviewed subject. Reviews by Ulmer (1971) and Cable (1972) provide a good historical survey as well as a treatment of trematode species which do not parasitize humans or their domestic animals. For more recent reviews, the reader is referred to Saladin (1979) and Christensen (1980).

Wright (1959) was the first to propose a sequential behavior pattern for trematode miracidia attempting to locate a host. In step one, the miracidium utilizes environmentally derived behavioral cues such as light and gravity. Its responses to these stimuli generally parallel the responses of the host, thereby placing the miracidium in the same region of the environment as the host. In step two, the miracidium moves randomly within the host environment, presumably scanning for the behavioral cues which will initiate step three. In this step, chemical cues facilitate host-selection and host-penetration by the miracidium. One behavior pattern may be inserted before Wright's number one. This is a dispersal phase seen in some species. Mason and Fripp (1976) showed that as Schistosoma

mansoni miracidia age, their linear swimming rate decreases and angular speed increases. These miracidia may be actively dispersing from the hatching site prior to the initiation of host-finding behavior. Additional support of the concept of dispersal behavior may be found in reports of trematode species whose larvae show reversal of responses to particular environmental cues, such as light, as they age (Isserof 1964). The initial response favors dispersal, while the reverse response favors host location.

Host-location by trematode miracidia has received considerable attention. Saladin (1979) has counted published reports of 176 miracidium-snail combinations. Although there have obviously been tremendous numbers of "parasitologist-hours" devoted to this subject, a full understanding of the basis of host-location by even a single trematode species remains beyond our grasp. The behavior of miracidia is probably best understood in schistosomes at this point in time. At least it has been the most thoroughly studied. Newly hatched S. mansoni miracidia are photopositive and geonegative (Chernin and Dunavan 1962). The responses of S. hematobium miracidia are temperature dependent but closely parallel the responses of the host snail at similar temperatures (Shiff 1969, 1974). There is some evidence that the photoresponse of S. mansoni miracidia is also temperature dependent (Mason and Fripp 1977a).

Chemical cues seem to be of paramount importance to the schistosome miracidium in host-finding. Indeed, there is some evidence that this type of cue may override responses to environmental stimuli. S. mansoni miracidia released at the water surface are able to infect snails at a depth of two meters (Prah and James 1978). Therefore, the phototactic and geotactic behaviors of these miracidia are not absolute. Although the chemosensitivity of trematode miracidia is no longer in doubt, the nature of the chemical cue(s) remains a source of controversy. Laboratory studies have reported behavioral responses to inorganic ions such as magnesium (Stibbs et al. 1976, Roberts et al. 1978) and magnesium/calcium ratios (Sponholtz and Short 1976), ammonia (Mason and Fripp 1977b, Mason 1979), free amino acids (MacInnis et al. 1974, Mason and Fripp 1977b), certain neurotransmitters, including serotonin (Roberts et al. 1978), and fatty acids (Wilson and Denison 1970, Mason and Fripp 1977b, MacInnis 1965, Bennett et al. 1972). Water which has held host snails is itself a powerful chemoattractant (MacInnis et al. 1974, Wright and Ronald 1972); however, laboratory studies have not attempted to use concentrations which approximate those of field conditions. Indeed, the snails in these studies are subjected to extremely crowded conditions. The "odor bouquet" of a stressed animal is often quite different from that of an unstressed animal. This observation has not been taken into account by investigators working with snail-conditioned water.

The chemoattractiveness of such a wide variety of compounds is somewhat puzzling. In addition, Roberts, Ward and Chernin (1979) have determined that miracidial responsiveness to snail-conditioned water is dependent upon encountering gradients where there is an abrupt decrease in concentration. Saladin (1979) proposes on the basis of miracidial receptor morphology that both organics and inorganics play a role in host-finding. Inorganics and perhaps free amino acids may serve as chemical cues which mediate the distance chemoreception and orientation phase of host location. Organics may be primarily penetration stimuli, perceived by contact chemoreceptors. Since the natural environment of the miracidium contains numbers of organisms other than the host snail, such a sequence of chemical cues may be essential for accurate localization of the proper host. Indeed, other species of snails as well as non-molluscan species can interfere with miracidial host location (Chernin and Perlstein 1971, Etges et al. 1975, Upatham and Sturrock 1973, Christensen et al. 1976, Christensen et al. 1977).

The actual proficiency of the miracidium as a "hunter" is not clear. There is no study which has quantitatively determined the percentage of miracidia which actually penetrate snail hosts in field conditions. There is some evidence that there is no correlation between snail density and snail infectivity rates (Chernin and Dunavan 1962, Chernin 1974). Since it is not within the scope of this paper to discuss host-parasite interactions which may provide a partial explanation of these observations, the reader is referred to Christensen (1980). Besides the presence of non-host organisms in the environment, the miracidium must contend with water turbidity (Upatham 1972, Christensen et al. 1978), velocity (Shiff 1968, Upatham 1973, James and Prah 1978), and temperature (Prah and James 1977)--all of which can decrease host-finding capability outside of optimum range of the particular parameter.

The ultrastructure of sensory receptors of the S. mansoni miracidium has been thoroughly examined by Pan (1980). There are six types of sensory organelles present. The miracidium has four multiciliated deep-pit nerve endings which are found at the four corners of the apical gland, an ideal location for contact chemoreceptors. Such multiciliated pit nerve endings have also been described in Fasciola hepatica (Wilson 1970). The schistosome terebratorium also has uniciliated nerve endings adjacent to the multiciliated pits. One-third of the way down from the terebratorium are three types of sensory organelles which lie in close association--the lateral papilla, the multiciliated saccular sensory organelle, and the multiciliated shallow-pit sensory papilla. The two lateral papillae are located one on each side of the miracidium and may function as "depth sensors" (Brooker 1972, Pan 1980). The saccular sensory organelles, likewise paired, have been suggested to be photoreceptors (Brooker 1972), gravity receptors in Fasciola hepatica (Wilson 1970), and vibration-sensing organelles (Pan

1980). The middle of the miracidium is girdled by about 12 multiciliated sensory papillae, which are spaced about 12 microns apart, and each contains 12 sensory cilia (Pan 1980). These may well be chemoreceptors. It is apparent from the above description that there are nearly as many functions ascribed to the various receptors as there are investigators who have studied them. Clearly, functional and not comparative morphological studies are imperative. Perhaps as detailed miracidial behavioral responses become better described, behavioral manipulations such as selective ablation of receptors by microsurgery will permit the accurate pairing of receptor and function.

SPOROCYSTS

In nearly all trematode species the (mother) sporocyst develops in the foot or mantle tissue of the snail, not far from its site of penetration as a miracidium. In the successive generation, the daughter sporocyst or redia undergoes a migration from the foot to the internal tissues of the snail, generally the hepatopancreas and ovotestis. For Schistosoma, at least the initial stage of this migration involves active penetration of the mantle tissue (Pan 1965). Using an albino strain of snails, Etges et al. (1975) observed schistosome daughter sporocysts migrating in the mantle wall, apparently using their anterior tegumentary spines to aid in locomotion. These daughter sporocysts subsequently enter extensions of the hemocoel, passively reaching the hepatopancreas by way of the hemolymph circulation. Since the daughter sporocyst demonstrates an active migration to the hemocoel, it is reasonable to predict that mechanical and chemical cues mediate this behavior. Muftic (1969) described intramolluscan stages of S. mansoni which respond in vitro to substances, probably steroids, produced by the snail ovotestis. Unfortunately no follow-up studies have been conducted. There are no reports of tegumentary sensory receptors in any trematode intramolluscan larval stage. Obviously, our current understanding of the behavior of intramolluscan stages of trematodes is weak.

CERCARIAE

Newly emerged cercariae generally exhibit responses to environmental cues which place them in proximity to their host. They may be either photopositive or photonegative depending on where in the environment their host is found, e.g. those with bottom-dwelling hosts tend to be photonegative and geopositive and vice versa. This does not always hold true however (Kennedy 1979), indicating that environmental cues may not be the principal stimulus for host location.

Host location by trematode cercariae has received only a fraction of the attention paid to that behavior in miracidia. Using computer analysis, Dixon (1980) has analyzed film footage

of cercarial host location by two species of trematode: Cercaria kirkstallensis, whose cercariae penetrate a chironomid larva, and Echinoparyphium recurvatum, whose cercariae use a snail host. The C. kirkstallensis cercaria is slow-swimming. However, upon entering a "zone of stimulation" around its host it increases its step speed and angle of turn. Dixon interprets this behavior as a "lie in wait" host-location strategy used by a cercaria which is much slower-moving than its host. When E. recurvatum enters the "zone of stimulation" around its snail host, it decreases its mean speed and increases its mean turning angle. This is a pursuit strategy used by a cercaria whose host is relatively slow-moving. Whether this classification of host-finding behavior will hold true for other trematode cercaria remains to be seen.

Cercariae show a positive response in a thermal gradient (Stirewalt 1971). Cohen, Neimark and Eveland (1980) found that S. mansoni cercariae respond to thermal changes as small as 2° C. These investigators were unable to determine whether cercariae actively follow a thermal gradient or random movement brings them into an area of higher temperature where they remain.

Penetration stimuli for schistosome cercariae have been well studied. Although temperature appears to be important in retaining the cercaria in close approximation to its host, the principal penetration stimuli are chemical in nature. Wagner (1959) found that S. douthitti cercariae failed to penetrate mouse ears which had been extracted with ether, but they shed their tails and discharged the contents of their penetration glands in response to the residue from the extraction. Free fatty acids elicited a similar response. Clegg (1969) found that the chemical cue for penetration by Austrobilharzia terrigalensis cercariae is cholesterol, the principal sterol of bird skin. S. mansoni cercariae show responses to butyric acid, aspartic acid, and glutamic acid (MacInnis 1969), as well as lipid extract from skin (Stirewalt 1971). The response of these cercariae to chemicals is enhanced by increased temperatures (MacInnis 1969, Clegg 1969). Like schistosome miracidia, the cues used by cercariae in locating hosts may be interfered with by the presence of other aquatic organisms (Christensen 1979).

The morphology of cercarial photoreceptors has been well described for ocellate cercariae. Short and Gagne (1975) described a photoreceptor in the cercaria of S. mansoni. There are three types of ciliated nerve endings described in cercariae of S. mansoni (Nuttman 1971). Ciliated cavities are located laterally on the body. These cavities contain five or six cilia and are innervated by a single nerve. Such a sensory organelle may be chemoreceptive. Cercariae also have two types of unciliated nerve endings--sheathed and unsheathed. Nuttman has proposed that both types are mechanoreceptors and that the unsheathed type may be chemoreceptors as well. How such a

receptor would work physiologically was not explained. The anatomical location of anterior uniciliated sensory structures around the openings of the penetration gland cells suggests a functional relationship between the two (Kemp and Powell 1970). Indeed, in a similar arrangement in the miracidium of S. mansoni, a nerve fiber was observed to connect the sensory bulb to muscles encircling the penetration gland cells, suggesting that the sensory structure serves as a trigger for gland cell extrusion (Mount, unpublished). There are no reports of proposed thermal receptors in trematode cercariae.

JUVENILE TREMATODES

The phenomenon of migration is a behavior which is poorly understood for all animals which exhibit it, including trematodes. The behavioral cues used for site location by juvenile trematodes are unknown. Certain trematode species arrive at their final destination by very direct migration routes. Fasciola hepatica gets to the bile duct via the abdominal cavity, using penetration gland secretions in combination with ingestion of host tissue. Clonorchis sinensis can either follow a similar direct route or it may gain entrance to the liver via the portal system. Migratory behavior of medically unimportant species is ably reviewed by Ulmer (1971).

Juvenile schistosomes, known as schistosomula, demonstrate a complex migration pattern. After spending a minimum of two days in the skin of a mouse, a S. mansoni schistosomulum is carried passively to the lung via the blood vascular system. S. mansoni schistosomula appear in the lung even if they have been injected into the tail vein of the mouse rather than allowed to penetrate percutaneously (Miller and Wilson 1978). The worm then spends at least 72 hours in the lungs where it grows and becomes elongated (Wilson et al. 1978). Miller (1976, as cited in Wilson et al. 1978) concludes that worms exit from the lungs via the pulmonary veins and subsequently enter the systemic circulation. She hypothesizes that since the probability of passing into the hepatic portal system is 0.1 for an individual schistosomulum, a single worm may make many passes through the circulatory system before reaching the hepatic portal system. Wilks (1967), in contrast, hypothesizes that worms migrate to the hepatic portal system via the pleural cavity, diaphragm, and liver capsule. Irrespective of the pathway taken, the schistosomulum must recognize some cue which identifies the hepatic portal system as its final destination. Such a cue would most likely be chemical in nature, although site location by schistosomula has not been investigated. If chemical cues do mediate site selection by schistosomula, and if schistosomula reach the hepatic portal system over an extended period of time, as Miller's hypothesis implies, additional behavioral cues may be provided by those schistosomes already in residence. Some evidence in support of this idea is seen in Paragonimus kellicotti which can locate a single

conspecific in a cat lung (Sogandares-Bernal 1966). Thermoreception has not been studied in these developmental stages. Temperature gradients within the body provide potential cues for migrating schistosomes. The possibility also exists that the schistosomulum does not use any behavioral cues to identify the hepatic portal system but merely becomes trapped there when its increasing size prohibits making another round through the pulmonary circulation (Wilson et al. 1978).

ADULTS

Mate selection in adult flukes has been superficially examined in schistosomes. This behavior is particularly important to this family of trematodes since they are the only dioecious flukes and spend their entire adult lives in copula. In addition to sperm, female schistosomes depend on the male for a certain protein which only he can synthesize (Atkinson and Atkinson 1980) as well as protection from damage by host immune responses (Kemp, unpublished).

Adult schistosomes respond to chemical and mechanical stimuli for mate selection. The relatively high frequency of homosexual (male-male) pairings seems to suggest that the principal mating stimulus for males is mechanical or thigmotactic (Armstrong 1965). However, female schistosomes are chemically attracted to males in the absence of tactile behavior (Imperia et al. 1980). Interestingly, this study reported a lowered response to increasing concentration of "pheromone"; that is, worms responded with greater intensity to the odor of one male than to the odor of three males. This may be attributed to a "window" effect in stimulus concentration where behavioral responses can be elicited only by a narrow concentration range. If pheromones prove to play a role in mating behavior, manipulation of this chemical cue may permit life cycle disruption analogous to insect pheromone systems.

Behavioral cues in site selection by adult trematodes is nearly a virgin research area. Fecal extracts have been found to be chemoattractants for schistosomes, particularly mated pairs (Awwad and Bell 1978). Since adult schistosomes do not come into direct contact with host feces, the significance of such behavior remains to be elucidated. Adult schistosomes are also reported to be attracted to a wide variety of indole derivatives in vitro (Khan 1964, as cited in Schwabe and Kilejian 1968).

No functional morphology studies exist for the described sensory receptors of adult flukes. Morris and Threadgold (1967) describe a ciliated receptor in Schistosoma mansoni. They hypothesize that this receptor is a tangoreceptor which would facilitate orientation in a fluid medium (blood) by selecting direction. However, the morphology of the receptor does not appear to support this hypothesis.

CONCLUSIONS

While there has been considerable progress in the area of trematode behavior, our knowledge remains superficial. Some agreement regarding the standardization of procedures and reagents needs to be reached. The obvious short-term goals of future research should deal with the definitive correlation of a function with each type of sensory structure and identification of specific host components which provide behavioral cues. The area of intra-host migration is virtually virgin territory and yet may be one of the most important fields of study in the future, particularly in those parasites which afflict humans and their domestic animals.

Another exciting area is the cellular and molecular biology of trematode chemoreceptors. Studies related to receptor molecules, their insertion in the surface membranes of sensory structures, the nature of the interaction between the receptor and signal molecule, and signal transduction may provide a model system which can interface with similar studies currently being conducted in cell biology and immunology.

The possibilities of controlling parasitic infections by interfering with chemotactic processes is attractive and theoretically doable. Such interference with extra-host stages would offer the best chances of control. Interference with site location within the host might also be of some use in controlling infections. However, a chemotactically confused migrating parasite might cause more damage than one which successfully locates its niche.

The field of study dealing with behavioral cues in trematodes, while still in its infancy, is an exciting area with extraordinary potential for both basic and applied scientific progress. Multidisciplinary collaboration in approaching questions raised by trematode behavior is essential due to the complex nature of the systems involved. Coordinated efforts by animal behaviorists, sensory physiologists, cell biologists, membrane biologists, molecular biologists, and biochemists will be needed if the problems outlined herein are to be adequately approached.

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Addendum to Formal Presentation by Dr. Kemp:

One of the major interests of our research program is schistosome membranes and membrane receptors. Our most recent work has been on membrane dynamics and on membrane receptors which bind to the Fc region of IgG molecules and to fragments of C3 of the complement system. Our working hypothesis is that the interplay between membrane dynamics and these receptors functions in aiding the parasite to escape the host's immune response. These studies have led us to develop an interest in membrane receptors which may have a function in parasite behavior.

It has been shown in immunological systems that Fc and C3 receptors directly influence the behavior of immune system cells. Certain immune effector cells are stimulated to initiate phagocytic behavior when their Fc receptors become associated with antigen-antibody complexes. In other systems cells are stimulated by Fc receptor bound antigen-antibody complexes to release chemical indicators which are chemoattractants to other immunocompetent cells.

The virgin lymphocyte has both monomeric IgM and IgD on its surface, each in association with a specific Fc receptor. It is the interaction between the macrophage, helper T cells, and these Fc receptor-associated surface immunoglobulins on the B-cell which initiates specific cell proliferation and antibody production.

Complement receptors also function in the facilitation of phagocytosis. Perhaps more relevant to our current discussion is the role played by elements of the complement system in chemotaxis. While the best known chemotactic component of that

system is the C5-7 complex, C3a and various fragments of C5 have been shown to enhance the migration of immune effector cells into an area of antigenic insult or inflammation.

Dr. Andre Capron and his colleagues at the Pasteur Institute in Lille, France, have demonstrated the presence of Fc and C3 receptors on the schistosomula of S. mansoni. Our laboratory at Texas A & M has demonstrated the presence of those same receptors on adult schistosomes. We believe the normal in vivo state of the parasite's surface is to be coated with host components, a considerable amount of which is immunoglobulin bound to Fc receptors. There appear to be two systems of parasite control over these adsorbed surface molecules. One system is characterized by a normal metabolic turnover rate, which occurs about every 2.5 hours. The other system is a rapid, specific, induced shedding phenomenon, which is initiated when the surface immunogobulin in question is bound to its specific antigen, a second antibody, or other appropriate ligand. The shedding activity can be blocked with sodium fluoride, 2-deoxy-D-glucose, cytochalasin B or D, or caffeine, indicating that the mechanism is glycolysis-dependent and microfilament-mediated.

Our working hypothesis for these studies is that at the molecular level all receptors, whether immunological or behavioral, are controlled by the same mechanisms. The receptor molecule is inserted into a membrane so that the specificity end is exposed to the external milieu. The affector end of the molecule is exposed to the cell's cytoplasm and is in association with the cytoskeleton, second-messenger systems, and energy source. A signal molecule would bind to the specificity end of the receptor, changing the energetics of the receptor-membrane relation leading to the leakage of cations across the membrane and the triggering of the second-messenger system. The compromised receptor is then controlled by the cytoskeletal system so that it may either be shed into the external milieu or endocytosed, either of which would cause a cessation of signal from that receptor molecule. By such controls the parasite would be able to sense the chemical nature of its environment and alter its behavior accordingly.

DISCUSSION

CASTRO: You said that the odor bouquet might be different in a stressed versus a non-stressed host. If you are using the term stress from a physiological standpoint, how would you define stress in an invertebrate?

KEMP: There are population stresses generated by placing animals together that are not used to being closely confined. These animals will respond differently than they would under other conditions.

DEVINE: Part of that type of stress is osmotic stress. Snail-conditioned water is often prepared by placing several hundred snails in half a liter of distilled water. I think you can see obvious osmotic stress in that type of situation. In lower vertebrates, stress induces changes in chemical signals used in social behavior. These signals may not necessarily be pheromones, but may be ordinary metabolic products.

BONE: Many types of snails possess alarm pheromones. The measurement of their effects on physiology is difficult, but you do observe avoidance reactions. They may also choose to bury themselves in the presence of stressful factors.

BENNETT: I was very intrigued by your studies on the inhibition of induced shedding by cytochalasin B and D and caffeine. We have been studying the outer surface of the schistosome electrophysiologically with respect to the interaction of these drugs with the membrane. We find that cytochalasin B in concentrations as low as one micromolar will cause drastic morphological changes in the surface of the schistosome and will alter the conduction of certain ions across the membrane. However, with caffeine we have never been able to detect much of a physiological change except at much higher concentrations where we find minor elevations of cyclic AMP and GMP. The antischistosomal drug praziquantel produces morphological disruptions similar to cytochalasin D. We currently believe that praziquantel and other similar antischistosomal compounds act synergistically with the immune response by affecting the tegumental membrane and rendering it more susceptible to immune attack. You intimated that there was a behavioral response to the activation of the Fc receptor. What kind of behavioral response have you seen?

KEMP: Induced shedding.

BENNETT: A physical shedding. I was thinking more in terms of some change in motor activity.

KEMP: We performed a study where we immunized infected mice against red blood cells to test for the presence of heterospecific antibody on the surface of the parasite. When schistosomes from these mice were incubated with red blood cells, the Fc receptor associated surface antibody bound to the red blood cells, attaching them to the worm's surface. The area of the tegument immediately beneath the bound red blood cell displayed considerable activity and movement. This activity characterized that particular area of the tegument only; the cytoplasm beneath non-red blood cell bound surfaces appeared quite placid by comparison. After about 15 minutes the red blood cell floated away and the unusual activity ceased.

DESPOMMIER: Are membrane components shed with the complex?

KEMP: At this point we do not know if the Fc receptor is shed with the antibody.

ROBERTS: I gather that your working hypothesis is that the surface of the schistosome is something like the surface of the lymphocyte.

KEMP: Yes, except I would say that it is more analogous to a macrophage or, to be consistent, with the syncytial nature of the tegument, a giant cell.

DAMIAN: In regard to the question of the similarities of the schistosome tegument and the lymphoctye, Dr. Gitter in my laboratory recently demonstrated the presence of MHC gene products on the adult parasite surface. However, he was unable to observe stimulation of allogeneic lymphocytes. This means that although these host antigens are present, they are not displayed in the same manner as they are on the lymphocyte.

BONE: Is there patching or capping on the parasite surface and is there actin in the tegument?

KEMP: We have observed patching and capping inconsistently. I suspect that those processes do occur, but because of dilution problems, either of the antibody or the antigen, we have not been able to definitively document them. As to your question concerning actin, we are currently gearing up to try to localize actin in the tegument using the heavy meromyosin technique.

BAILEY: You commented that the location of the schistosome in the liver might not be due to positive attraction, but merely to the entrapment of the parasite in the portal system. Do you know of any evidence for this hypothesis?

KEMP: No. That was an hypothesis put forward by Miller. I do not find the idea particularly attractive myself, but I cannot argue against it.

BENNETT: In regard to that question, it is of interest to note that serotonin concentration along the mesenteric veins and in the portal veins is about twice as high as in the rest of the circulation. S. mansoni cannot convert tryptophan to 5-hydroxytryptamine, and since serotonin is a stimulant, perhaps the parasite is cueing on that molecule to locate its particular niche. Since S. hematobium is found in the pelvic veins, perhaps it has retained the ability to make serotonin. It is a testable hypothesis.

DAMIAN: You mentioned the observation that substances in the feces are chemoattractants. These substances could be bacterial products which are taken up by the intestine. For S.

hematobium there might be something in the urine playing a similar role.

LONG: Perhaps we should consider this in another way. Maybe flukes reach their sites in order to escape from some unpleasant environment. We have talked a lot about attractants. Perhaps they are simply getting away from something they find antagonistic.

DESPOMMIER: Going back to the cercariae, will you speculate on the practicality of using compounds which stimulate penetration responses in the field?

KEMP: By using slow-releasing attractants, one might bring cercariae to one place for subsequent destruction. Alternatively, one might develop chemicals which would short-circuit the behavioral response, causing early release of the penetration gland cells. However, if our working hypothesis is correct, the sensory receptor mechanism would probably be the same for most aquatic organisms, particularly the invertebrates. The use of such a substance might then disrupt the ecosystem. An attractant specific only for miracidia or cercariae would seem to be the best means of control. It is of interest to note in this regard that there are naturally occurring cercariacidal compounds released by plants. There are long stretches of certain rivers in South America kept free of schistosomes because of the growth of such plants near the water. Also, many of us are aware of the cercariacidal properties of cedar-chip bedding, which we used for a while in our mouse cages.

ROBERTS: We performed some studies in Eli Chernin's lab concerning the prevention of snail infection. We were unable to interfere with snail infection using a slow-releasing magnesium compound. In retrospect, I think that just getting to the host is only part of the problem for the parasite. There is actually a whole complex of sensory behaviors that lead to the successful location, penetration, and establishment of the parasite within the host. It is that complex of behaviors which imparts the specificity leading to the successful finding and penetration of the host. That is probably why simple schemes of dumping magnesium slow-release compounds into streams in endemic areas may never be successful.

KEMP: To carry that idea to the molecular level, perhaps receptor function requires inorganic and organic components. You may actually have two binding sites on one receptor molecule and both must be occupied in order to trigger a response. The problem is most certainly more complicated than we have been able to visualize to this point. There are some very bright people who have spent considerable amounts of time trying to unravel the problem, with very little success.

BAILEY: The migration of flukes such as Fasciola to the liver is an interesting phenomenon. Although on primary infection flukes show a random distribution and migration to the liver, flukes from subsequent infections appear to head straight for the liver. They seem to be following some track laid down by the previous worms.

KEMP: Frank Sogandares did some interesting work with Paragonimus kellicotti. A single parasite took about two weeks to migrate to the lungs and encyst. However, a second parasite placed in the host after the first worm had established itself took only a couple of days to reach the first worm in the lungs. He speculated that either a pheromone or circulating antigen-antibody complex from the cyst formed a gradient which the second parasite followed. If larval Paragonimus have Fc receptors, perhaps he was right.

ROBERTS: One of the difficulties with following a gradient of a diffusable chemical is that gradients tend not to be stable. Embryonic tissues apparently migrate to specific places by following gradients that are laid down on substrates, not diffusable gradients. I think that is an interesting observation that may apply to parasites like Paragonimus and Fasciola.

BEHAVIORAL AND PHYSIOLOGICAL CUES OF CESTODES, WITH PARTICULAR REFERENCE TO SEROTONIN (5-HT)

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INTRODUCTION

The digestive system is concerned with intake of nutrient material, secretion, digestion and absorption of nutrients required by the animal, motility of intestinal contents, and elimination of waste material. To that end there are regional differences in the structure and function of the gastrointestinal tract, and one can consider the intestinal mucosa and lumen as offering a sequential series of potential niches within which parasites can become established and reach sexual maturity. Not surprisingly, considering the range and choice of niches available, the gastrointestinal tract is the most favored location for adult cestodes and for metazoan parasites in general.

The characteristic complex mucosal structure of the vertebrate intestinal epithelium, while offering a variety of morphological attachment sites for a parasite, reflects the polyfunctional role of the intestinal epithelium and contrasts with other epithelia as the gall bladder, kidney, and Malpighian tubules and salt glands and with cestode tegument, which are all primarily transporting surfaces (Mettrick 1980, Mettrick and Podesta 1974).

The second and major variable for an intestinal parasite is exogenous food recruitment and the changing physical and chemical parameters of the intestinal mucosa and lumen that follow from feeding. It is among these parameters that one is most likely to find possible agents or cues that influence the behavior and physiology of any intestinal parasite, including tapeworms.

GASTROINTESTINAL TRACT

In a vertebrate the process of feeding activates the digestive process, which is then controlled through vagal-hormonal

regulation (Go and Summerskill 1971). The three components involved are the gastrointestinal tract, the endocrine system, including the GI hormones and amines, and the neural system, mediated through vagal and sympathetic enervation; intestinal parasites add a fourth interactive component (Mettrick 1973, 1980). The complexity of the vagal-hormonal regulatory process is illustrated in figure 1, representing stimulatory and inhibitory routes involved in gastric secretion.

In the intestine itself the prime activities are secretion, digestion, absorption, and motility. The release of acid chyme from the stomach into the duodenum greatly increases the $[H^+]$, and therefore the luminal pCO_2 also rises significantly, following the reaction

$$H^+ + HCO_3 \rightleftharpoons H_2CO_3 \rightleftharpoons H_2O + CO_2 \cdot$$

The resulting effects on the four activities of the intestine are illustrated in figure 2 (see Mettrick 1973, 1980, Mettrick and Podesta 1974 for references).

The addition of the parasite Hymenolepis diminuta to this dynamic interactive system results in changes in the intestinal nutritional (Mettrick 1971a,b, 1972) and absorptive gradients (Podesta and Mettrick 1976a, 1977), in absorption (Podesta and Mettrick 1974a,b,c, 1976a,b), and in the physical and chemical parameters of the intestinal lumen (Mettrick 1971a,b,c, 1975a, b, Dunkley and Mettrick 1977). Attempts to correlate the migratory behavior of H. diminuta to one or more of the many intestinal gradients that can be demonstrated have been equivocal, and the cues to initiation of anterior and posterior phases of the worm's response remain elusive (Hopkins and Allen 1979, Arai 1980). The observation that oral and intravenous treatment with the intestinal hormones and amines, cholecystokinin, secretin, histamine, and 5-hydroxytryptamine, induces a migratory response by H. diminuta (Podesta and Mettrick 1981, Mettrick and Podesta 1982) focused attention on the intestinal endocrine system in general and on 5-HT in particular as possible sources of the environmental cues influencing worm behavior and site selection. Electrical vagal stimulation, simulating the effect of host feeding on gastrointestinal function mediated via the vagal nerve, induces rapid gastric secretion (Cho et al. 1976) release of 5-HT and cholecystokinin from the proximal intestinal mucosa and of pancreatic secretions (Konturek et al. 1974, Ahlman et al. 1976a,b, Schwartz et al. 1978), and also results in an anterior migration by H. diminuta (Mettrick and Cho 1981b). The migratory response is enhanced when the pylorus is ligated to prevent gastric secretion from entering the duodenum.

5-HT IN THE GASTROINTESTINAL TRACT

Enterochromaffin (EC) cells, found in the mucosa throughout the

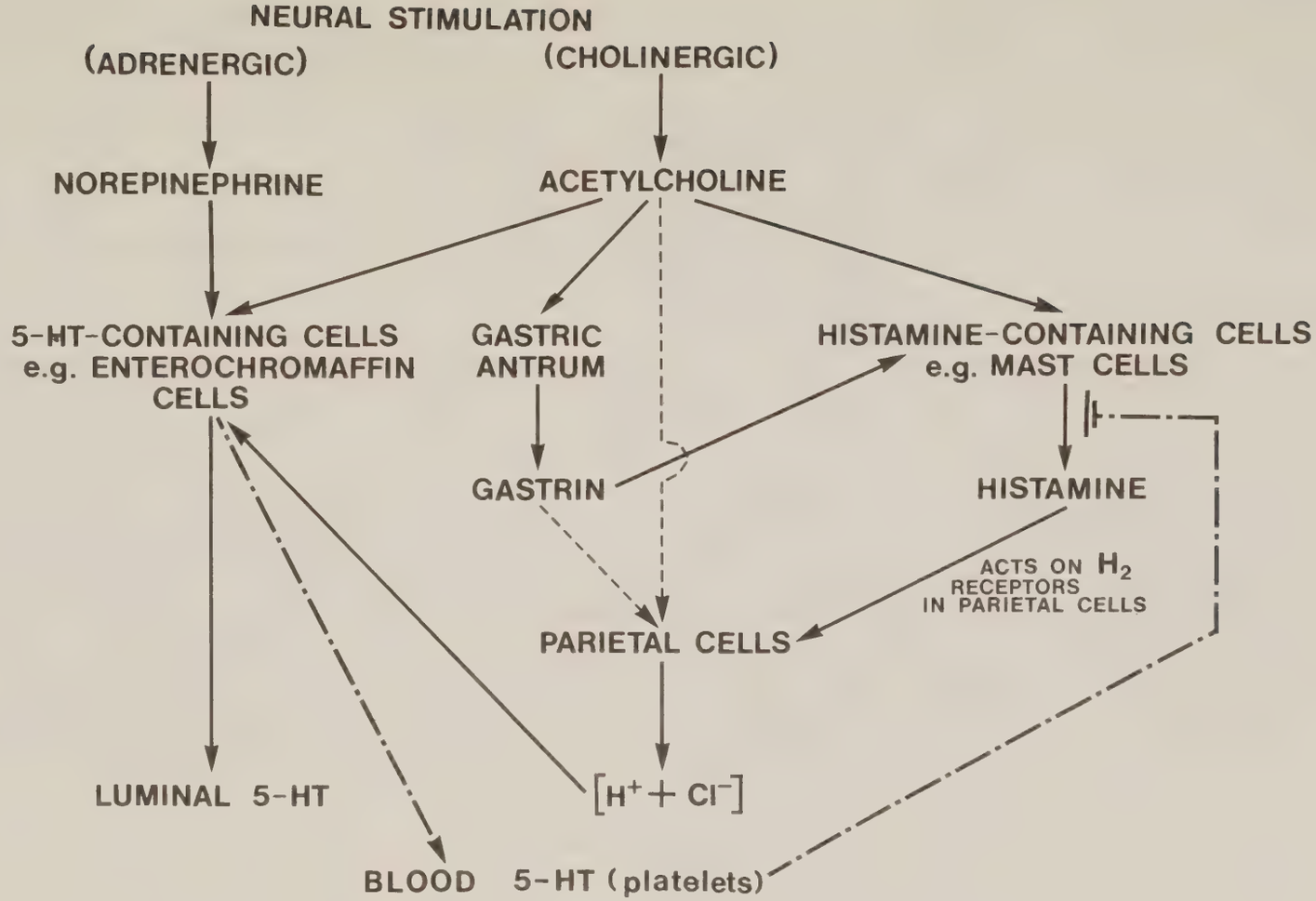

Figure 1.--Neural-hormonal-amine interactions involved in gastric acid (HCl) secretion. The dotted lines indicate a possible action of Ach and gastrin on the parietal cells.

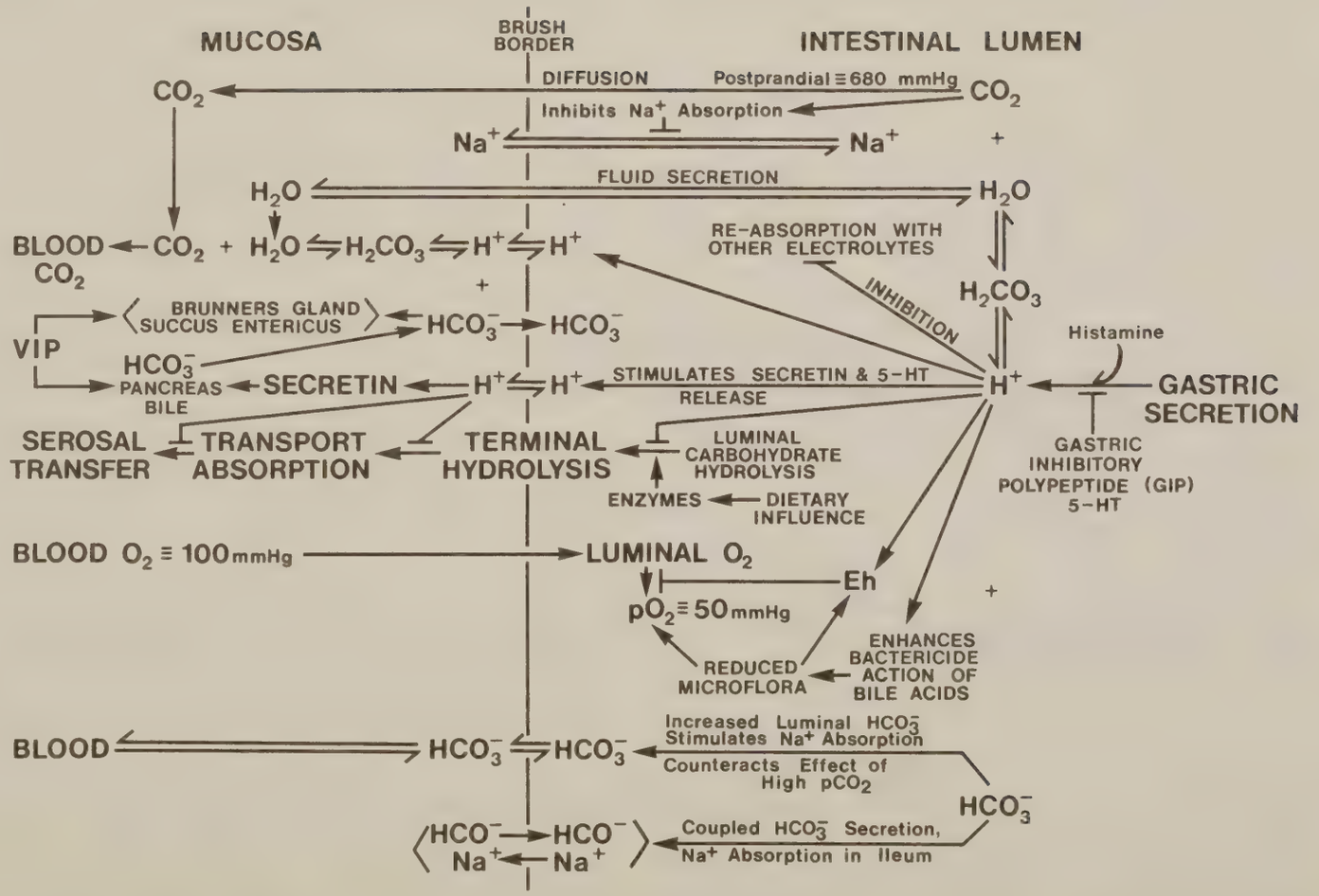

Figure 2.--Luminal-mucosal fluxes and interactions.

gastrointestinal tract of all mammals and being most numerous in the proximal intestine, are the major source of 5-hydroxytryptamine; the digestive system accounts for 60% of the total body 5-HT content (see Thompson 1971, Pettersson 1979). The EC cells synthesize and secrete 5-HT into the intestinal lumen; some is taken up by the mesenteric veins. In rats and mice 5-HT is also found in mast cells (Benditt et al. 1963, Erspamer 1966); it arises by both de novo synthesis from tryptophan and by passive and active uptake of the amine itself. Mast cell 5-HT is bound to histamine at specific binding sites, and it is suggested that 5-HT release from the mast cells is passive, being secreted along with the histamine. Serotonin is not normally found in the enterochromaffin-like (ECL) cells. The third source of GI 5-HT is the β cells of the pancreas.

The EC cells are part of the intestinal endocrine system and as they extend into the lumen are believed to react to luminal stimuli (Fujita and Kobayashi 1973). The following mechanisms for control of the secretion of 5-HT have been demonstrated:

1. Neural control involving both adrenergic (Tansy et al. 1971) and cholinergic (Tobe et al. 1976) nerve fibers of the vagal nerve (Pettersson 1979).

2. Luminal stimulation by hypertonic glucose and acidification of the duodenal lumen (Drapanas et al. 1962, Resnick and Gray 1962, Fujita and Kobayashi 1971, Kellum and Jaffe 1976b).

3. Increased intraluminal pressure and peristaltic wave pressure following feeding.

4. Circulating substances such as the catecholamines (Burks and Long 1966, Ahlman et al. 1976a).

The physiological role of 5-HT in the intestine of a normal animal is complex and includes the following:

1. Serves as potent inhibitor of gastric acid secretion (Black et al. 1958, Jaffe et al. 1977).

2. Produces vascular response to 5-HT (Dolani et al. 1970); required for the vasodilatory effects of cholecystokinin and secretin.

3. Decreases hepatic glycogen and inhibits hepatic gluconeogenesis.

4. Stimulates intestinal smooth muscle and is implicated in neural-hormonal control of intestinal motility.

5. Increases levels of plasma adenosine 3',5'-phosphate.

6. Inhibits protein synthesis in the intestine (Majumdar and Nakhla 1979).

7. Influences insulin release (Lechin et al. 1975, Lebobitz and Feldman 1973).

5-HT AND MIGRATIONAL BEHAVIOR OF HYMENOLEPIS DIMINUTA

Following intraperitoneal, intramuscular, subcutaneous, and oral administration of 5-HT, the distribution of both scolices and biomass of H. diminuta within the small intestine changes significantly. As would be expected, parenteral administration of 5-HT results in a slower and less marked anteriad migratory response by H. diminuta than that following oral treatment. There is also a relationship between the intestinal level of 5-HT and the extent of the migratory response; the higher the luminal 5-HT concentration the more marked the worm's migratory response (Mettrick and Cho 1981a).

Increasing the dose of the 5-HT treatment results in increasingly higher 5-HT levels in the intestinal lumen and in the arterial carotid circulation. With increasing luminal 5-HT levels there are also increased 5-HT levels in worm tissue and a more rapid, greater, anterior migratory response. The pattern of 5-HT levels in worm tissue closely follows changes in luminal 5-HT concentration, which in turn reflects the oral dose administered, supporting the suggestion that worm 5-HT is of extrinsic host origin rather than endogenous de novo synthesis by H. diminuta (Bennett and Bueding 1973, Mansour 1979).

Two further studies on the behavioral responses of H. diminuta to changing levels of luminal serotonin provide strong evidence that the relationship is direct. The circadian migratory behavior of H. diminuta is well documented (Read and Kilejian 1969, Tanaka and MacInnis 1975). Cho and Mettrick (1982) have correlated the pattern of circadian worm migration with a similar circadian variation in the levels of 5-HT in worm tissue, the intestinal lumen, the intestinal mucosa, the intestinal food content, and the time in the feeding cycle. The 1600-h stage in the circadian cycle marks the initiation of host feeding, which is then followed by steadily rising 5-HT levels in both the intestinal lumen and worm tissue. The higher the worm 5-HT levels the more marked the migratory response. Fasting reduces luminal and worm 5-HT levels and eliminates the normal luminal circadian 5-HT increase at 1600 h; there is also no migrational response by H. diminuta. While confirming the link between food intake by the host, leading to increased luminal 5-HT levels and a migratory response by the worms, the experiments did not, however, rule out the possibility that other stimulating agents, also secreted under vagal control, may be involved.

Earlier studies have shown that feeding and worm migration are

linked, irrespective of the time in the normal circadian cycle, if the host is first fasted for a short period (Mettrick 1971a,b, 1973). Administration of the specific 5-HT antagonist methysergide hydrogenmaleinate and of the nonspecific blocking agent magnesium sulfate to fasted, infected rats resulted in a 90% and a 67% inhibition, respectively, in the normal worm migratory response (see table 1). These results confirm a direct relationship between 5-HT and worm migratory response and expand further on the now considerable evidence for changes in the levels of 5-HT in the intestinal lumen and circulating arterial blood which can be related to the pattern of host feeding. Mettrick and Cho (1982) have also demonstrated that, although 5-HT secretion is predominantly in the proximal small intestine (Erspamer and Testini 1959, Thompson 1971), because of the posterior flow of intestinal contents, the intestinal 5-HT gradient increases from the proximal to the distal small intestine.

There is evidence that high magnesium levels reduce the release of 5-HT from the enteric nerves and possibly from other tissues in the intestine (Gershon and Erde 1981). The antagonistic effect of magnesium on 5-HT has been demonstrated in the ileal longitudinal smooth muscle of guinea pigs (Singh 1974, Goldstein and Zsoter 1978) and in isolated arteries and veins of rabbits (Goldstein and Zsoter 1978). Magnesium also reduces the sensitivity and maximum response normally shown to acetylcholine, angiotensin, and KCl (Altura and Altura 1971). The mechanism by which magnesium alters the normal contractile response to 5-HT has not been determined, although an interaction between Mg^{2+} and Ca^{2+} has been suggested (Mettrick and Cho 1982).

Methysergide is a specific 5-HT antagonist (Douglas 1976), and

Table 1.--Effect of 5-HT inhibitors on the migrational behavior of *Hymenolepis diminuta* (after Mettrick and Cho 1982)

Treatment	Migratory index (see Podesta & Mettrick 1981)	Inhibition %
2 ml H_2O + food	0.463	0.0
1.2 mmol $MgSO_4$ + food	.154	66.6*
$1.2x10^{-3}$ mmol methysergide + food	.098	78.8*
$1.2x10^{-2}$ mmol methysergide + food	.045	90.3*
$1.2x10^{-2}$ mmol methysergide, no food	.034	92.7*
Untreated controls, no food	.000	100.0*

* $P<0.001$ compared with water-treated group.

the 90% inhibition of the normal in vivo migratory response of H. diminuta in the presence of methysergide confirms the direct role of 5-HT in worm motility. Mettrick and Cho (1982) also suggested that the luminal 5-HT gradient, which increases in concentration from the proximal to distal ileum, may be involved in the determination of the direction of the worm migratory response. The suggestion is based on the effect of 5-HT on individual worms: First, those worms lying posteriorly in the intestinal lumen, in a high 5-HT concentration, will have a higher 5-HT tissue level and show a more rapid and enhanced migratory response than anteriorly positioned worms in the duodenum/jejunum, due to the dose response effect of 5-HT on worm migration. Second, as individual worms in 15-day-old, 10-worm infections are approximately 30 cm long, 5-HT levels in worm tissue will show a gradient matching the luminal 5-HT levels. Worm 5-HT is localized around the nervous system, which would facilitate the transmission of information on 5-HT levels from the tail to the scolex, thus enabling the parasite to determine the direction of the 5-HT gradient and to respond by migrating anteriorly. The importance of the "tail" of H. diminuta in determining the luminal position of the worm has already been demonstrated (Hopkins and Allen 1979). Mettrick and Cho's (1982) hypothesis provides an explanation for the importance of the tail.

Irrespective of the possibility of the luminal 5-HT gradient's being a directional cue, the anterior phase of the migration of H. diminuta is up the luminal glucose gradient (Dunkley and Mettrick 1977) so that the role of 5-HT in stimulating glucose uptake becomes increasingly effective as worms move anteriad (Mettrick et al. 1981). A corollary of the related role of 5-HT in the regulation of worm carbohydrate metabolism is that the higher 5-HT levels of worm tissue in the distal small intestine will increase gluconeogenesis by the worm, thus ensuring the availability of energy for the anterior migratory response.

Acetylcholine is a neuromuscular inhibitor in H. diminuta (Wilson and Schiller 1969). While it is possible that the posterior phase of the circadian migration of H. diminuta is simply due to the fact that when worm 5-HT levels decline below the threshold level of response the worms drift posteriorly with the intestinal contents, from a biological point of view a more reliable control system seems likely. Increasing levels of acetylcholine in the worms, due to inhibition of the appropriate cholinesterases, would ensure the inhibition of contractability, allowing the worms to be passively carried posteriorly in the gut due to the normal flow of intestinal contents. Serotonin and acetylcholine would therefore be acting as antagonistic agents in the overall pattern of the circadian migratory response.

5-HT IN THE CIRCULATORY SYSTEM

A rise in circulating 5-HT after a meal has been demonstrated by Kellum and Jaffe (1976a) and Mettrick and Cho (1982). There is also a circadian rise and fall in 5-HT levels in arterial (carotid) blood related to the normal 24-h feeding behavior of an animal (Cho and Mettrick 1982). In vivo vagal stimulation increases plasma 5-HT levels in the portal veins by 250% after only 15 min of stimulation (Pettersson 1979) and also elevates circulating arterial 5-HT levels (Cho and Mettrick 1980, Mettrick and Cho 1981b).

While most of the 5-HT synthesized by the EC cells is secreted into the intestinal lumen, some reaches the mesenteric veins, where it is rapidly taken up by the platelets (Hughes and Brodie 1959, Born and Gillson 1959). Plasma 5-HT levels account for only approximately 5%-10%. The binding of endogenous 5-HT to the platelets bypasses the degradation of plasma 5-HT during passage through liver and lung (Thompson 1971). Guilbault and Froehlich (1974) have suggested that values reported for plasma 5-HT are the result of breakdown of platelets during the chemical determination of 5-HT and that there is no 5-HT in blood plasma.

As the highest concentration of 5-HT is found in the portal circulation (Erspamer and Testini 1959), Bennett and Bueding (1973) suggested that the localization of adult schistosomes in the mesenteric-portal venous system was due to the dependence of the worms upon 5-HT. This could also be applied to the location specificity of both Fasciola hepatica and Hymenolepis microstoma for the liver and bile duct, respectively. The concept of 5-HT having a role in either host or location specificity for blood parasites is strengthened by Catto and Ottesen's (1979) observation that the affinity constant (K_m) for 5-HT uptake by blood platelets is much lower than that for S. mansoni, which may be related to the higher concentration of 5-HT found in the mesenteric portal system. Secondly, because of the low 5-HT levels in blood plasma, the schistosomules, during their first 5-7 days in the final host outside the mesenteric-portal vessels, may be dependent upon their demonstrated ability to decarboxylate 5-HTP to 5-HT. The adult worms cannot decarboxylate 5-HTP and hence rely entirely on exogenous 5-HT from ingested blood platelets.

5-HT, GLUCOSE ABSORPTION, AND CARBOHYDRATE METABOLISM

Among trematodes, 5-HT has been shown to stimulate glucose uptake (Mansour 1959) and to affect the regulation of carbohydrate metabolism (Mansour 1962, Mansour and Mansour 1962, Stone and Mansour 1967, Hillman et al. 1974). Serotonin has a similar action on in vitro glucose uptake by H. diminuta, the rate of uptake being linear and parallel to that of 5-HT-free control groups but at a significantly higher level (Mettrick

et al. 1981).

In the absence of glucose in the incubating media, the presence of 5-HT significantly increases the rate of glycogen breakdown in H. diminuta, as has also been previously demonstrated in Fasciola hepatica (Mansour 1962). T. E. Mansour and colleagues (Mansour 1959, 1962, Mansour et al. 1960) demonstrated that, in F. hepatica, 5-HT activates glycogen phosphorylase and adenylate cyclase and increases the formation of cyclic 3',5'-AMP. In mammalian systems epinephrine, through the formation of c-AMP from ATP catalyzed by adenylate cyclase, activates phosphorylase kinase and increases the level of active phosphorylase (Sutherland and Roll 1960). Mansour et al. (1960) suggested that activation of phosphorylase, leading to glycogen breakdown in F. hepatica, follows the same route as in mammals except that 5-HT replaces epinephrine. While the activation of adenylate cyclase by 5-HT has been confirmed in Schistosoma japonicum (Higashi et al. 1973), c-AMP production stimulated by 5-HT has not been demonstrated in S. mansoni and Ascaris lumbricoides.

5-HT SYNTHESIS AND UPTAKE BY HELMINTHS

Synthesis of 5-HT involves a two-stage hydroxylation and decarboxylation of the amino acid tryptophan. In mammals the rate-limiting enzyme in 5-HT synthesis is tryptophan hydroxylase, which is activated by pyridoxal phosphate, leading to the production of the intermediary 5-hydroxytryptophan (5-HTP).

Tryptophan hydroxylase in helminths has not been identified (Mansour and Stone 1970) and, to date, no adult helminth has been shown to be capable of de novo synthesis of 5-HT from tryptophan, leading both Bennett and Bueding (1973) and Mansour (1979) to conclude that 5-HT found in helminth tissues is of extrinsic, host origin. Some helminths have been shown to be capable of the second transformation of 5-HTP to 5-HT (Kisiel et al. 1976).

A relationship between luminal and worm tissue 5-HT levels implies uptake of 5-HT across the parasite tegument. Evidence that H. diminuta can transport peptides (Mettrick 1975b) implies that transport of either or both 5-HTP and 5-HT should also be possible. Serotonin transport by Schistosoma mansoni has been demonstrated (Bennett and Bueding 1973, Catto and Ottesen 1979). Deamination of 5-HT by MAO (monamine oxidase) results in accumulation of 5-hydroxyindole acetic acid (Pletscher 1968.)

COORDINATING MECHANISMS IN HELMINTHS

As with free-living organisms, parasitic worms must be capable of adjusting to environmental changes; the question is, "How are the environmental stimuli mediated?" As previously

indicated, interaction of the neural and endocrine systems is a key factor in coordinating the functions of the digestive system. But in helminths, lacking both a circulatory system and a sophisticated nervous system, neurosecretion is the basic coordinating mechanism. Neurosecretory cells synthesize materials which are transported along nerve axons to a fiber terminal and there released. The aminergic neurons synthesize both catecholamines (i.e., dopamine, noradrenaline, adrenaline) and indolamines (i.e., tryptamine, 5-HTP, 5-HT). Neurosecretory activity has been demonstrated in a number of helminths (see table 2), with dopamine and 5-HT being the two most commonly occurring amines.

The cholinergic neurons are characterized by cholinesterases, and acetylcholine, a neurotransmitter, has been demonstrated in A. suum, S. mansoni, F. hepatica, and H. diminuta. Pharmacological evidence indicates that in trematodes Ach is an inhibitory neuromuscular transmitter; an increase in Ach levels depresses muscular activity (Barker et al. 1966), whereas cholinergic blocking agents stimulate motor activity (Bennett and Bueding 1971). Acetylcholinesterases are the predominant active enzymes found in the nervous system of H. diminuta and H. nana (Wilson and Schiller 1969); Ach also appears to be an inhibitory neuromuscular transmitter in H. diminuta.

Serotonin has been shown to act as a neurotransmitter in stimulating motor activity in a number of worms, including F. hepatica (Mansour 1957, Beernink et al. 1963), S. mansoni (Barker et al. 1966, Bennett and Bueding 1971, Hillman and Senft 1973), Mesocestoides corti (Hariri 1974), Taenia pisiformis (Mansour 1964) and H. diminuta (Mettrick et al. 1981).

The presence of a number of biogenic amines in helminths and, to a large extent, fulfillment of the basic requirements needed to demonstrate the action of neurotransmitters in a given group of organism (Johnson 1972) provide a strong indication that the amines have a role in coordinating both behavioral and physiological responses by helminth parasites. In the specific case of 5-HT the indicators support the view that the parasites depend on extrinsic 5-HTP or 5-HT. The indolamines and the catecholamines appear, therefore, to be prime candidates for further study, with the aim of being able to upset the coordinating mechanisms by which such parasites are able to respond to the normal stimuli of their environment.

ACKNOWLEDGMENT

I am indebted to the Natural Sciences and Engineering Research Council of Canada for research support.

Table 2.—Distribution of biogenic amines in helminths

Helminth	Amine	Reference
NEMATODA		
Phocanema decipiens	Noradrenaline Dopamine	Goh & Davey 1976a,b
Ascaris suum	Adrenaline Noradrenline	Bargiel et al. 1970
Spirometra larvae	5-HT	Tomosky et al. 1974
Aspiculuris tetraptera	5-HT	Anya 1973
Caenorhabditis briggsae	Noradrenaline Norepinephrine Epinephrine 5-HT	Kisiel et al. 1976
TREMATODA		
Fasciola hepatica	Dopamine Norepinephrine	Bennett & Gianutsos 1977 Chou et al. 1972
Schistosoma mansoni	Dopamine Norepinephrine 5-HT	Gianutsos & Bennett 1977 Bennett & Bueding 1973 Chou et al. 1972
S. japonicum	5-HT Norepinephrine Dopamine	Chou et al. 1972
S. haematobium	5-HT	Chou et al. 1972
Paragonimus westermani	Dopamine	Chou et al. 1972
Paragonimus ohiarae	Dopamine	Chou et al. 1972
CESTODA		
Hymenolepis diminuta	5-HT	Chou et al. 1972 Lee et al. 1978
H. nana	5-HT	Lee et al. 1978
Mesocestoides corti	5-HT	Hariri 1974

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DISCUSSION

CASTRO: How do you rule out the possibility that worm migration is not due to the gradients provided by the biliary pancreatic secretion?

METTRICK: The primary reason is that in the rat there is no gall bladder, and bile flow is continuous. Bile still flows if you starve the animal. You do not, however, get any migrational response by the worms, which suggests that bile was not the factor influencing worm migration.

BENNETT: Do you know the concentration of free 5-HT within the lumen of the gut of the rat?

METTRICK: We have measured luminal 5-HT levels under three conditions. First, in the normal circadian change in luminal 5-HT, the levels vary between 50 and 90 µg. In feeding experiments, luminal 5-HT increases to 18, 25, and 30 µg for the proximal, mid, and distal third of the small intestine. Third, luminal 5-HT levels up to 6.4×10^3 µg have been recorded in dose-response experiments. We have also measured mucosal 5-HT levels in the stomach and small intestine, and, of course, worm tissue 5-HT.

BENNETT: The reason I asked is because if the concentration of 5-HT in the lumen is one or two micrograms per gram of intestinal contents, I don't know how much of that is really available. You're dealing with about ten micromolar 5-HT. Now in your physiological experiments you're dealing with one millimolar 5-HT. The question I have to ask is how relevant is one and two millimolar in in vitro experiments, where you expose parasites to rather large amounts of 5-HT, to the free concentration of 5-HT that actually exists within the lumen of the parasite. Basically what I am asking is what effects do micromolar amounts of 5-HT have upon certain biochemical parameters such as increased lactate production or worm motility, etc? You showed us what one and two millimolar 5-HT do.

METTRICK: First, in the studies on circadian changes in 5-HT in the intestine, animals are normally fed and the 5-HT levels are not experimentally induced. Second, there is evidence (Lee et al. 1978, J. Parasitol. 64: 257-264) that worm 5-HT is localized around the nervous tissue; there could similarly be high concentrations of 5-HT at the sites of glucose uptake, or

of enzyme activity much higher than the basal 5-HT value expressed as μg/g of worm tissue. Of course I don't know if this is the case, but the localization of 5-HT distribution in H. diminuta provides support for the hypothesis. In current studies, we are looking at the threshold levels of 5-HT required for a physiological or biochemical change, but again we don't know localized tissue on intercellular 5-HT levels.

BENNETT: With one millimolar 5-HT you're generating a tremendous amount of muscular activity, which, of course, would require greater glucose utilization and, thus, increased lactate production under aerobic conditions. If you did it under anaerobic conditions, you see the alternate pathways which you very clearly indicated. Do you have any proof to show directly that 5-HT inhibits the enzyme pyruvate kinase? Have you purified the enzyme and then added 5-HT and demonstrated inhibition by 5-HT? What about phosphofructokinase and hexokinase?

METTRICK: Pyruvate kinase is significantly inhibited by 5-HT. The enzyme was partially purified, but certainly not to homogeneity. Hexokinase is the one in which there was not a marked response. I think that is probably due to the extraction procedure which we had to use, involving magnesium. Magnesium has already been shown to be a blocker of 5-HT action. What I think we finally got was the enzyme plus magnesium already bound to it. Not surprisingly, it did not have any reaction when 5-HT was added.

BENNETT: One last question. You showed that methysergide had an effect on the migration of the parasite. As 5-HT caused increase motility of the gut itself, was intestinal transit affected?

METTRICK: Both Mg and methysergide inhibit 5-HT-stimulated intestinal transit and, by implication, gut motility.

BENNETT: Did you use something that would inhibit gut motility but not have an effect on 5-HT, such as photavin?

METTRICK: No. We haven't tried that, nor something to inhibit the effects of histamine.

ROBERTS: I have two questions. The first is more general variation of Dr. Castro's question: How do you know that 5-HT is not simply a transduction molecule and that it in some way affects the motility of the worm and that there is not actually a more specific molecule that is a behavioral attractant? The second question: Isn't your 5-HT luminal gradient backwards? If it isn't backwards, isn't it true that worms are actually repelled by 5-HT rather than attracted?

METTRICK: In answer to your first question, it is very difficult to prove the negative, which in this case means proving

that only 5-HT is involved. That we have not done. Your point is well taken, as very recently polypeptide "P" has been described in the gut closely associated with 5-HT (Ahlman et al. 1981a, 1981b). Our evidence for 5-HT being the modulator is circumstantial, but it's supported by the concomitant physiological and biochemical actions of 5-HT on the worms. With regard to your second point, the intestinal 5-HT gradient increases from the proximal to distal region. Anterior migration by the worms is therefore moving down the intestinal 5-HT gradient to a lower concentration and, at the same time, the worms move up the intestinal glucose gradient. As we have not been able to correlate worm migration patterns to luminal glucose gradients, I am suggesting that the anterior migration is related to 5-HT and that the response to the glucose gradient is, at best, only secondary.

Let me pursue the hypothesis further. There is presumably a threshhold level of 5-HT required for a worm migrational response. A worm moving forward in the gut may move into a region where the 5-HT concentration is below that threshold response level. Neuro-muscular action stimulated by 5-HT is then inhibited or ceases, so that the worm might then be carried passively posterior due to the general flow of intestinal contents. From a biological point of view, I would suggest that there is a more reliable mechanism to ensure that the posterior phase of the migratory response does occur. A possible mechanism might involve acetylcholine, which, in H. diminuta, inhibits neuro-muscular action and is therefore antagonistic to the effect of 5-HT. If worm acetylcholine-esterases were inhibited (perhaps by increased worm tissue H^+ concentration), then worm acetylcholine levels would increase, preventing muscular contraction. As a result, worms would be carried, passively, posterior into a higher 5-HT luminal concentration and the cycle would recommence.

ROBERTS: What is the evidence for a luminal 5-HT gradient in which the high end is distal and the low end is proximal?

METTRICK: On one of the slides, I showed the increasing luminal levels of 5-HT following feeding. In the duodenum an asymptote for luminal 5-HT was reached in 30 minutes; similar asymptotes in the mid-intestinal region and in the ileum required a longer time after feeding, and the asymptotes were at increasingly higher levels as you go down the gut. This is explained in terms of maximum mucosal 5-HT secretion occurring in the duodenum, which is then carried posteriorly due to intestinal transit.

KEMP: It seems to me like you're in a particularly enviable position being able to localize receptors in your worms, if indeed those are receptors, by using radiolabelled 5-HT and then identifying the receptors in which they are associated.

METTRICK: I agree, and that is why I was particularly fascinated by Dr. Bennett's presentation yesterday.

KEMP: Are there more receptors toward the posterior end of the worm or toward the anterior end of the worm, or do you know?

METTRICK: We don't know, but it is likely that there are a similar number of receptors on each segment of the tapeworm, based on the fact that all segments originate just behind the scolex and then enlarge during maturation. We are particularly interested in trying to correlate the neurophysiological aspect of worm migration to the behavioral cues, physiology and biochemistry--in short, an integrated interpretation of what is involved.

KEMP: Just one more thing. Working with a system like this is very interesting from the standpoint that it looks like you're going to have maturational differences between the anterior and posterior regions of the worms. It seems to me you're going to have more receptors toward the posterior than you would to the anterior or would you have less?

METTRICK: In the anterior part they would be closer together, so you would have more per given surface area of the strobila. In the posterior region, as the segments grow, individual receptors in the tegument may become further apart.

FRANDSEN: This seems like an obvious question here, but I don't think I have heard it discussed. What effect is there on neuroreceptors in the strobila as the segments mature? It seems like an interesting area to be looked into structurally.

BENNETT: The question is whether there is a receptor gradient along the length of the strobila. I did some work about ten years ago on hymenolepids and 5-HT. Most of the 5-HT is located in the anterior region. But that would be moved down the strobila of the worm as new segments are formed. You can't really say what is the physiological significance of lower 5-HT concentration when, in fact, it could be localized in the areas that are quite critical to the function of the individual segments. I would think that physiological methods could easily determine the physiological importance, at least with respect to the impact of 5-HT on muscle in various regions. I would suspect that the anterior region of the parasite is probably the most sensitive to 5-HT.

FRANDSEN: It would be interesting to determine if there is a metabolic rate gradient as one goes from the anterior region of the strobila through the mature region to what is essentially a tissue made up of eggs.

METTRICK: The gravid region is metabolic because of all the

eggs and developing embryos. We have never looked at regional effects of 5-HT down the strobila.

DESPOMMIER: I'm curious as to the conclusions based on whether you're dealing with a single worm moving up and down or whether you're dealing with groups of worms moving down and up at the same time. You mentioned that you were concerned about how they were distributed along the gut in terms of gradients and 5-HT.

METTRICK: Why do these worms migrate at all? The answer, of course, is that the migration is related to worm nutritional needs and to the ability of the worms to maximize their competitiveness against mucosal nutrient uptake by the host. The most important nutrient required is glucose, and I suggest that the carrying capacity of worm biomass in an infection is related to available glucose. Just as there is competition between host mucosa and worm tegument for available luminal nutrients, so too is there competition between individual worms in a multi-worm infection. In the case of H. diminuta, individual worms are not all tightly bunched together; by serial overlap a ten-worm infection utilizes the entire length of the small intestine (approx. 100 cm), although individual worms are only some 30 cm long when fully relaxed.

Consider the most anterior worm and the most posterior worm in a ten-worm infection. The luminal glucose gradient decreases rapidly from proximal to distal; the 5-HT gradient is the reverse. The anterior worm will, therefore, have a low 5-HT tissue concentration, but high glucose and glycogen levels; the posterior worm is the opposite. The posterior worm, therefore, has a higher demand for luminal glucose than the anterior worm. There is a further point; in the parasitized gut H_2O secretion is enhanced and fluid absorption inhibited, which results in more liquid intestinal contents, facilitating worm movement.

I have suggested that the luminal 5-HT gradient is reflected in the worm tissue gradient and that, as a result, the 5-HT concentration in the scolex is lower than that in the terminal segments of any particular worm. As 5-HT is localized around the nervous system, it seems reasonable to conclude that a worm could determine that there was a difference in 5-HT concentration between scolex and "tail" through the effect of 5-HT on nerve excitation. The anterior migration would decrease the level of excitation in the "tail," which would also explain the importance of the "tail" as demonstrated by Hopkins.

Going back to the difference between the anterior and posterior worms, the latter, being in a higher 5-HT concentration, will show a higher degree of motility than the anterior worm and, as a result of the relative positions of all worms in a multiworm infection, will change not only with regard to their location in the intestine but also with each other. Think of it as a

stock-car race with worms passing each other or being overtaken on each circuit, with a worm-circuit being the 24 h circadian migration.

DESPOMMIER: If there's only a single worm present, does it drop back?

METTRICK: Well, when you have a single worm present, you don't get nearly as large a migrational reponse, and the individual worm is, of course, very much larger in length and biomass.

BONE: If you could change the slope of the intestinal 5-HT gradient, could you not condense or disperse your worm population?

METTRICK: If our theory is true, yes.

BONE: I have been intrigued for years by the reports regarding selfing and cross insemination in which selfing in tapeworms could not be carried beyond six or seven generations. The interesting thing here is that if you change that 5-HT slope up and down you could, I would think, spread or condense the population. You could, therefore, regulate the population--assuming it's not random. Is there a greater likelihood of cross insemination in the midpoint of that regression line than there would be on th extremes of that regression line? I would think the population would be outbreeding in the center and inbreeding at the limits? Does that make any sense?

METTRICK: Yes. I see your point. But you would have to maintain constancy because the contents of the lumen are moving posterior, which is what creates the gradient in the first place.

LONG: Are there any factors which would change that? Can you make the luminal 5-HT gradient more shallow?

METTRICK: Possibly if one can inhibit 5-HT secretin from the enterochromaffin cells.

LONG: Are there any natural conditions?

METTRICK: Normally MAO, released by the intestinal bacteria, reduces luminal 5-HT through catabolism. But in the parasitized gut the luminal bacterial flora is greatly reduced, so the natural regulation of 5-HT intestinal levels breaks down. This possibly explains why luminal 5-HT levels are much higher in parasitized than in normal uninfected animals.

BEHAVIORAL CUES IN MIGRATION AND LOCATION OF PARASITIC NEMATODES, WITH SPECIAL EMPHASIS ON *TRICHINELLA SPIRALIS*

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INTRODUCTION

Nematode parasites occupy a wide variety of niches within the vertebrate host (for a review of the niche concept as it relates to parasitism see C. R. Kennedy 1976). Little is known, however, about the specific signals which guide them to their niches. Most nematodes reside only as adult worms in their definitive host. In contrast, some worms, such as the filariae and *Trichinella spiralis*, live within the same host as both adults and larvae. Hence, these parasites are ideal organisms for studying site-selection behavior, since the adult and larval stages of these latter groups often occupy different niches simultaneously. The discussion to follow will focus primarily on the site-selection behavior of *T. spiralis*.

T. spiralis lives two distinct lives, one enteral and one parenteral. The worm can infect all mammalian hosts, and, in general, each stage occupies the same niche within them. The enteral phase of the infection takes place largely in the intramulticellular niche (i.e., within the columnar epithelium) (Wright 1979). Stages L_1 through L_4 and the adult male and female worms live there, while the migrating L_1 and the infective L_1 stages live parenterally within the cytoplasm of the Nurse cell (Purkerson and Despommier 1974).

ENTERAL PHASE

The enteral phase begins with the host's ingestion of the infective L_1 larva in its Nurse cell. Following digestion in the stomach the larva becomes freed from the surrounding tissue and is now ready to enter the small intestine. Experimentally induced infections are usually initiated with the pepsin-HCl isolated infective L_1 larva, and therefore the behavior of this stage has been frequently observed.

This stage exhibits coiling and uncoiling movement but does not locomote in a typical snake-like fashion. This movement is classified as type-one behavior. Shortly after entering the small intestine, the infective L_1 larva enters the enteral niche (figure 1). If worms are removed from the host at this juncture, their movement is typically serpentine and is called type-two behavior (Sukhdeo, personal communication).

The cues from the host's environment which trigger the change from type-one to type-two behavior are as yet unknown; speculation as to the precise causes for this change revolve around host-induced alterations of the infective L_1 larva cuticular surface (Despommier, in press). When the in situ infective L_1 larva is treated with 1% pepsin-1% HCl for 1 hour at 37° C, then examined under the electron microscope, the outer cuticular surface is observed to consist of four layers. These same four layers have been observed in situ (Despommier 1975). Pepsin-HCl isolated worms are relatively impervious to harsh chemicals, including 2% glutaraldehyde. Such larvae have been observed to remain alive in 2% glutaraldehyde for up to 1 h

Figure 1.--L_1 larva of Trichinella spiralis entering its intramulticellular niche within the columnar epithelial cells of the small intestine. The drawing is based upon electron micrographs, courtesy Dr. K. Wright.

(Despommier, unpublished observation). However, if these worms are then subjected to 0.25% trypsin at pH 8.2 for 1 h at 37° C, layers 3 and 4 are removed (figure 2), and the worm is more susceptible to fixation in glutaraldehyde. Similarly, Berntzen (1965) and Sakamoto (1979) were able to grow the infective L_1 larvae of Trichinella spiralis through four molts to the adult stage only after treating them with trypsin and pancreatin. Apparently, the removal of layers three and four enables the worm to perceive that it is in a suitable environment for continued growth and development. Electron microscopical evidence confirms that at least layer four is removed during the first 3 to 6 h after the infective L_1 larva enters the columnar cells (Wright, personal communication).

Site selection within the small intestine by the infective L_1 larva of T. spiralis has also been investigated extensively with interesting results. For instance, it is common to observe in most rodent hosts that an incoming infection locates within the anterior half of the small intestine (Gursch 1949, Larsh and Hendricks 1949, Podhajecky 1962, Campbell 1967, M. W. Kennedy 1976, and Sukhdeo and Croll 1981). Exceptions abound, however, with ICI female mice and young mice (i.e., 2-4 wk old) of most other strains harboring worms further down in the gut (Larsh and Hendricks 1949, Campbell 1967, and Denham 1968). The volume of fluid in which an experimentally induced infection is initiated also dramatically affects the location in the gut in which the worms take up residence (Sukhdeo and Croll 1981). These investigators found that the larger the fluid volume, the further down in the gut the infection would be established. Worms could be made to take up residence in the anterior-most portion of the gut if the gut's smooth muscle is relaxed with diphenoxylate and atropine just prior to infection. Furthermore, if the infecting dose is placed in a lower-than-normal location within the gut (i.e., by infecting a ligated loop of gut), then the worms develop to adults there and remain in place (Sukhdeo and Croll 1981).

Trichuris muris, another member of the Trichurata, usually resides in the caecum of mice but will establish in the ileum if caecectomy is performed prior to infection (Panesar and Croll 1980). T. muris lives in a syncitium of columnar cells which cover only the stichosome region of the worm (Lee and Wright 1978). After ten days of development, T. muris cannot be transplanted successfully to another host, which suggests that the ability of the worm to induce its intramulticellular niche is irreversibly linked to its early morphogenesis. This is not the case with T. spiralis, since adults can be transplanted at will from host to host without apparent loss of infectivity (Katz 1960, Matoff 1961, 1963).

Following enteral site location by T. spiralis, the worms molt four times in rapid succession (Villella 1958, Ali Khan 1966, Kozek 1971). Upon maturing to adults, they mate--usually with-

Figure 2.--The cuticular surface of a pepsin-HCl isolated infective L_1 larva after digestion in 0.25% trypsin at pH 8.2 for 1 hour at 37 °C. Note that layers 3 and 4 have been partially degraded and lifted off from the surface. x115,500.

Figure 3.--A migrating L_1 larva (arrow) penetrating the muscle cell. x1,600.

in the first 6-12 h after the last molt (Hemmert-Halswick and Bugge 1934, Wu and Kingscote 1957, Gardiner 1976). The sex ratio of male to female worms has been shown repeatedly to be between 1:1.5 and 1:2 (Boyd and Huston 1954, Podhajecky 1963, Denham 1968). Since all females become inseminated during the course of infection, it follows that the males must mate more than once. Hence, male worms site locate more than once while in the intramulticellular niche.

Studies on pheromone attraction among male and female adults have given some insight as to the possible mechanisms for mating behavior and, consequently, site location. Bonner and Etges (1967) and Belosevic and Dick (1980) have conducted in vitro studies which clearly showed that female worms produce a water-soluble substance(s) which serves as a sexual attractant. An in vivo correlate for the functioning of a sex pheromone system has been recently documented by Sukhdeo and Croll (1981). In these studies, unisexual and bisexual infections of infective L_1 larvae were initiated and the distribution patterns in the small intestine of each recorded. Female infective L_1 larvae were distributed throughout the anterior half of the small intestine similar to the distribution of the bisexual infection. In contrast, male infective L_1 larvae exhibited a random pattern of distribution. The latter findings suggest that males actually produce a substance or substances which repel other males. Such a repellant could serve to randomize further the distribution of male worms in the gut, thereby maximizing their chances for encountering female worms. In vitro studies by Belosevic and Dick (1980) are in agreement with this concept, but Bonner and Etges (1967) found the opposite results.

PARENTERAL PHASE

Newborn L_1 larvae are produced within five days after mating (Denham and Martinez 1970) and shortly thereafter migrate to and penetrate (figure 3) their parenteral intracellular niche, the striated skeletal muscle cell. The route of migration to this site is largely through the lymphatics (Basten and Beeson 1970, Harley and Gallicchico 1971). The migratory L_1 larva of T. spiralis does not discriminate among cell types as to which it will or will not penetrate, and in this respect it is like the protozoan Toxoplasma gondii. However, once within cells (figure 4), worms apparently perceive recognition signals provided by the host cell, since the worms are able to migrate out of unsuitable cells and resume their journey to an appropriate one. The nature and number of intracellular cues responsible for such site-selection behavior are just beginning to be discovered.

Experiments aimed at defining the specificity of site selection by the newborn L_1 larva have been carried out by Hughes and Harley (1977). By use of in vitro migration chambers these workers were able to show that the newborn L_1 larva was posi-

Figure 4.--The larva of Trichinella spiralis migrating within the sarcoplasm of the striated skeletal muscle cell during its early phase of growth and development within its intracellular niche. This drawing is based upon photographs obtained from synchronously growing worms.

tively attracted to a -120 mV stimulus. These results suggest that the electrical potential of a contracting muscle fiber (i.e., -120 mV) attracts the worm to it. The newborn L_1 larva was repelled by a -90 mV electrical potential. Similarly, concentration gradients of KCl also repelled the worms. Finally, none of the internal components of striated skeletal muscle (i.e., phosphocreatine or glycogen) was effective in either attracting or repelling larvae. Unfortunately, these studies have not been extended.

Once the worm enters a striated skeletal muscle cell, it remains there, inducing a series of fascinating changes in the architecture of the cell (Despommier 1975) and directing its morphogenesis into the Nurse cell.

In addition to the site-selection behavior of T. spiralis just described, another tropism needs to be mentioned and discussed briefly, namely its ability to detect and migrate towards a

thermal gradient. Thermal gradient migration of nematodes was first noted by Ronald (1960), using Terranova decipiens, but was more fully exploited by McCue and Thorson (1964, 1965), using Nippostrongylus brasiliensis. In these latter studies, it was shown that N. brasiliensis would migrate toward the heated end of a migration chamber, even to the point of dying if the temperature at the hottest end exceeded lethality. This investigation further showed that thermal migration response was greatly diminished if N. brasiliensis was derived from a host which was in the process of immune rejection (McCue and Thorson 1965). The L_2-L_4 larval stages and adult stages of T. spiralis have also been shown to migrate toward the heated end of a solution of 0.85% NcCl (Despommier 1973). Furthermore, as with N. brasiliensis, T. spiralis adults migrate more sluggishly in a thermal gradient if they are obtained from an immune host (Despommier and Campbell, unpublished observation).

The neurological basis for site selection by T. spiralis has not been studied, but electron micrographs obtained from random sections of worms of some stages strongly indicate that it possesses nerve tissues which may function as receptors of environmental stimuli (McLaren 1976, Wright 1980, Despommier, unpublished observations). Those nerve tissues associated with the anterior regions of the esophagus are particularly suggestive of this kind of function.

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DISCUSSION

BOTTJER: The layers three and four are present on the developing L_1. Is their formation necessary before that L_1 can become infective?

DESPOMMIER: Well, it's never absent on infective L_1 larvae.

BOTTJER: Has anybody ever taken an L_1 without layers three and four and tried to start an infection?

DESPOMMIER: Good point. Yes, it was thought that layers three and four might allow it to pass through the stomach unscathed. Because it's resistant to pepsin digestion, one would think that perhaps it has some survival characteristics for the worm. So I digested some pepsin-isolated worms in trypsin and then tried to infect mice. They infected beautifully. It has absolutely no effect on the worm's ability to survive through the stomach. As a matter of fact, the only stage of the worm that can't survive going through the stomach is the fourth stage larva. For some reason, L_4 around 24 to 28 hours after infection does not survive via the oral route. Worms removed at that time and transplanted orally would not take. This is the work of Frank Katz at Seton Hall University. If you take any other stage (i.e., the L_1 through L_3 or the adults), some of those worms can survive through the stomach and take up residence in the small intestine. So layers three and four are apparently not important in terms of one's survival through its environment. I think they are functional strictly in the Nurse Cell. They are quickly removed once the worm enters the small intestine and are probably non-functional at that point.

BOTTJER: Can you take a newly penetrated L_1 into a muscle cell and remove it after eight hours or even at three or four hours and get that larva to start up a new infection?

DESPOMMIER: Well, a newly penetrated muscle larva would actually be a migratory stage worm. It doesn't have layers

three and four. They only develop after the 12th day of intracellular life in the muscle.

BOTTJER: Can this larva infect before three and four is formed?

DESPOMMIER: Another good question. In other words, when is the worm pepsin-resistant after it develops? Almost all investigators using Trichinella have taken infections from orally-induced infections which are obviously non-synchronous. The larvae that are produced from those infections are all of a unique age. They may be microseconds apart in age, but they are uniquely different. And so when you ask the question when is the earliest time point that the worm after reaching the muscle can now be infective for another animal, you're asking that question about a very few worms. If you synchronize the infection from intramuscularly-induced newborn L_1 larvae, then you can ask the question for the whole population of worms, and the answer emerges. Between day 14 and 15 is the time point at which the larvae are now infective for another animal. It's after layers three and four are laid down. But it's at the same time that granulogenesis is occurring in the stichocyte cells. It's another issue that I didn't plan to discuss.

BOTTJER: Is there still no definite evidence whether layers three and four are host or parasite derived?

DESPOMMIER: No experiments have been done to explore whether or not these layers are host-derived, or parasite-derived, or both. Some membrane-like layers around different parasites are derived from each. And it's possible that this is one of those examples, because work with ferritin-labeled antibodies and fluorescent antibody work done by other researchers all indicate the outer surface of the infective L_1 larva is antigenic. Recently, Parkhouse, Ogilvie and others have also shown that the surface contains antigen. They are really looking at the outer surface of layer four, which is the inner surface of the Nurse Cell, and it contains two parasite antigens. It's probably got a lot of host components also. This issue is just emerging as a problem, and we have to find out more about this.

CASTRO: When you say that the worms paired in the epithelial tissue, does that pairing occur in the larval stage? I take it by the fact that the males can mate more than once that they must be able to back out and go in again and find another female, moving in and out of that tissue.

DESPOMMIER: That's correct. It must be "gangbusters" around 30 hours. In this case, if you have 1,000 larvae that you have infected the rats with, and they are all in, say, the first 10 centimeters of intestine, it must be like one enormous orgy. Within six hours all the females are mated. That can only occur if the males are coming in and out of the tissues.

CASTRO: Would you find some of them pairing up within an hour or so?

DESPOMMIER: No. Gardner indicated that it took about 25 hours before he saw evidence of a pair of worms he could actually say was a male and female. You can tell a male worm after 14 hours by scanning electronmicroscopy. From that point on it gets more male-like until finally at the fourth molt it's a typical male. But what really has to be done is to segregate out the non-males or the females and incubate them in vitro in some kind of a pheromone-attraction system with normal males to ask, "When is the earliest time point in the molt cycle that the female begins to look like a female to the male?" I'm sure that experiment would tell you exactly when to look for worm pairs. Insemination does not occur before 30 hours. So if you take worms out after 30 hours, or 28 hours, which is just at the final molt, and you look for sperm in the seminal receptacles of the females, they are not there. Kozek, Ali Khan and Hemmert Hallswick and Bugge did this experiment, and all of them concluded that mating occurs between 30 and 36 hours. It's just a six-hour period, no more than that. Kozek did some additional studies which were difficult. It's like the Heisenberg principle of asking how many times can a female mate and how many offspring does she give off during that time. You can't do that experiment because you have to kill the animal to find out how many adults are there. And you also have to kill them 30 days later to find out how many larvae they produce. You can't kill the same animal twice. And as a result, we can't answer that question because you can't check the same animal to see how many other adults are there.

Another interesting experiment I was going to save for the discussion was the one of Campbell and Yaktis. They took a single infective L_1 larva and infected a mouse with it. A week later they infected the same mouse with another infective L_1 larva. Twenty-five percent of the mice acquired a viable infection in the muscle. You know the small intestine is a vast tissue in the eyes of Trichinella. The answer to the question of how many offspring does a single mating pair give off is about 600 worms. And that's about the limit of the infection from the terms of this reproductive potential. You could tell from the numbers based on that whether that female has mated more than once with more than one male.

KEMP: Darwin Murrell has done some very interesting labeling work--I'm not sure which nematode, but I know it wasn't Trichinella. In some other work with antibody and antigen, the same sort of shedding that we get with Schistosoma was seen. I was wondering if you had seen anything like that or do you know anybody who has in Trichinella?

DESPOMMIER: Shedding on the surface of the Trichinella?

KEMP: Yes.

DESPOMMIER: Yes. I know of some data on it, but I haven't seen them. I know of some being processed right now, suggesting that if you incubate the infective L_1 larva in a special medium, it will shed some of the particulate antigens. They call them particulate antigens, but I refuse to accept that term now that I know what the surface looks like. They haven't looked at the worm's surface other than with light microscopy. Diane McLaren is trying to prepare them for EM. She's had a difficult time fixing this material. But they claim they have a medium in which they can get constant shedding of cuticular material, and I find that somewhat bizarre because I think the amount present in the surface is finite. Once you remove the worm from the host, it's not going to be able to synthesize any more surface material. At least it doesn't seem as though it should be able to do it. My original thought was that antigen secreted from the stichocytes had been trapped on the surface after incubating the larvae in vitro. I sent them some of our stichocyte antigens, and they tried inhibition tests using their antiserum against the surface components. They were unable to inhibit their surface reaction using our antigens. Their results clearly showed we were dealing with two different kinds of antigens. I now accept the fact that there are surface antigens. A case for their role in protection is being made, but clearly they're removed by the host by trypsin shortly after entering the small intestine, and therefore I don't believe they play any role.

DUSENBERY: I have trouble seeing electrical activity of the muscle act as a stimulus to attract the worm.

DESPOMMIER: Me too. I just gave you the data.

DUSENBERY: I would suggest perhaps that the thermal response might be taken seriously as a means of detecting muscle cells. Active muscles probably have a little more than the surrounding tissue. And at least a few nematode species have been shown to be excessively sensitive to thermal gradients; fractions of a degree over the length of the worm can be detected at least by some species. That's a real possibility, I think.

DESPOMMIER: This is the only stage (i.e., the migratory L_1 larva) that hasn't been looked at in terms of thermal migration. Every other stage has been looked at and found to respond positively. So I don't know at this time if it was thermotaxis or not that attracted it to the muscle. But if it were, wouldn't you expect a lot more brain damage to occur from this infection if it was just that and nothing else? However, it is not typically seen in trichinellosis.

DUSENBERY: Well, yes; but there's a lot of electrical activity there too.

DESPOMMIER: I agree.

DAMIAN: What stage was it that was curtailed in the thermal gradient?

DESPOMMIER: The adult stage. The adult stage during immune rejection is a primary infection. What we were trying to do was to collect all adults from immune and non-immune animals to test whether or not their offspring are less infective. We had some results that suggested that worms derived from immune animals produced defective L_1 larvae. However, the main finding that emerged from the study was the fact that the adult worms just didn't migrate. They were very difficult to collect because they never got to the center of the circular thermal migrating device that we used.

FRANDSEN: It seems like the logical thing to look for would be positive response to lactate?

DESPOMMIER: Yes, that's right. There have been some disappointments I think in terms of the whole behavioral aspect of the Trichinella. They are easy worms to obtain. You can get as much as you want, and you can get as many different stages as you want. You can collect them in pure form and do virtually anything you want to with them. Surprisingly, nobody has really picked up on this. There are very few qualified behaviorists who have picked Trichinella as a model.

KEMP: As for the smooth muscle, does it have the same electrical activities?

DESPOMMIER: I don't believe it does. It doesn't penetrate smooth muscles.

KEMP: I was wondering if you gave a worm a choice between two muscles, might it be keying on it?

DESPOMMIER: I don't think it chooses the muscle. It doesn't choose the muscle that it should be going into at least in vitro. One of the things we thought about was whether or not contracted muscle might be necessary for larval penetration (i.e., whether there are some physical aspects of penetration as well as taxic aspects). A muscle consists of a tight bundle of cells. If it's undergoing contraction, this tends to squeeze all of the fluid out of it. It has to push everything in close together, and perhaps making this a part of the response of the worm. Maybe the larva senses this and can only penetrate when it has to push up against something else in order to get in. Maybe that's why our in vitro stuff didn't work. The larvae live for three weeks in cultures, but they just sit there and put their head ends on the surface of the muscle cells. Even in the chromaster muscle preparations where the muscle fibers can be made to contract, the worm will not

enter the muscle cells. We have observed about 20 of them in this condition. There was a report, I think from Kentucky, that they had gotten larvae to go into some kind of a muscle fiber preparation in vitro, but they could not repeat the experiment.

BOTTJER: I was just wondering if it would be possible to set up a millivolt of a 120 or 90 current through a tissue culture system in the muscle cell.

DESPOMMIER: Oh, yes; I've seen it. They do it all the time when they grow muscle and nerve together. They stimulate the muscle by the nerve.

BONE: What is the relationship between the capillary and the larvae? Is it packed in there?

DESPOMMIER: How big is the larva compared to the capillary? It's about the same diameter as the capillary. The larva itself is about 7-1/2 microns in diameter.

BONE: We have managed to get them to mate in the gut even held in the tissue bag, but when you open it, they're gone.

DESPOMMIER: We have had them in capillaries and observed them under the microscope. They don't just sit there. They move in a serpentine fashion. However, they don't penetrate, so we're going to have to look after that. If you leave the muscle preparation in its natural state, you won't be able to observe it. It would twitch all over the place. No matter how good a video tape set-up you have, you'll never be able to see what went on. It's another Heisenberg-type experiment. When you can see what goes on, nothing happens! If you leave it so you can't see what goes on, it would probably penetrate right away. We're at kind of a technical snag right now. It would be nice to observe penetration in these living muscle preparations.

CASTRO: I'd like to take Dr. Long's approach to the problem. They do leave the capillary and the myocardium; they'll invade the myocardial tissue and they don't encyst there. When you said they don't encyst in smooth muscle or they don't penetrate smooth muscle, is it that they don't encyst or actually don't penetrate? What I'm getting at is your approach; maybe that's just something unique about the muscle tissue. Is the skeletal muscle the thing that allows it to react?

DESPOMMIER: Oh, I agree.

CASTRO: A parasite is going there and possibly getting stuck. And then the reaction generates a so-called Nurse Cell, which may be a Nurse Cell, looking at it from the eyeballs of the parasite, because you did draw eyeballs on your diagram. But

it may be a host-defense reaction, a very specific and unusual type of host reaction.

DESPOMMIER: Yes, I like that idea. That's great.

CASTRO: It's not something that the parasite is going after. It just gets caught there.

DESPOMMIER: It's like a screen to remove the parasite from more sensitive tissue like nervous tissue. There has to be some signal to tell a parasite not to migrate back out again. None of those signals are given off in any other tissue--so it's got to have something that says, "You're in muscle cells now, stay here because you're going to live here for a long time."

CASTRO: Physiologically the skeletal muscles provide such a large mass of tissue in the body that you're not going to damage that particular organ function. Whereas if you've got it encapsulated in the myocardium or some smooth muscle capillary, venules or arterioles, that could be a problem.

DESPOMMIER: The nude mouse is a living example. We both know the results from that. A nude mouse can accumulate, from a 200 worm infection, about 300 to 400,000 larvae in its musculature. Its muscle tissue is solid larvae and Nurse Cells! It's just like little strings of pearls down the muscle fiber. The origin and insertion is in place, and there's a little bit of normal muscle in between each one of these things that's able to contract enough to allow it to feed, to walk, to breathe--all the normal functions. You would never guess that it was a different mouse except that it moves a little bit more slowly than the other mouse. But it's almost solid larvae. So I think you are right. It's a perfect relationship that the worm establishes once it gets inside the muscle cell.

CASTRO: It's called a Nurse Cell; and then you said that you really don't know anything about the nutrition?

DESPOMMIER: Other than from what Stewart and Reed have done. And they've done that preparation of diaphragm and percolated on top of that various nutrients and watched the incorporation into both the parasite and the host tissue. It's got to get through this Nurse Cell in order to get to the parasite. There's no other way for it to get there. And it recruits selectively, according to their data. The surrounding tissue doesn't take it up at the same rate that it does. So for those reasons alone we term it a Nurse Cell. It's not consumed by the parasite. It stays in its relationship for a long time. Its metabolic rate is much higher than the surrounding tissue even after it gets completely formed.

CASTRO: What's the metabolic rate?

DUSENBERY: The metabolic rate of the Nurse Cell-parasite complex remains high after its formation. The incorporation rates for all the protein precursors that they used are elevated even after the worm is fully grown, even after the Nurse Cells have fully grown.

CASTRO: It's not getting into the parasite?

DESPOMMIER: Some of it is. That indicates a very high turnover rate for some of these things, that's all. At least, that was their conclusion.

CASTRO: The evidence is not clear about the metabolic rate of the parasite--evidence about nutrients' getting into the parasite and being incorporated into the different tissues.

DESPOMMIER: A lot of things have to be done.

CASTRO: Yes, but there is no reason why the metabolism should be very active.

DESPOMMIER: There are data which indicate very strongly that both the host and the parasite component of some infections metabolize at a high rate. That's all I'm saying.

CASTRO: I don't know whether that says much about the parasite.

SITE FINDING AND SITE SPECIFICITY IN THE COCCIDIA

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FACTORS AFFECTING SITE OF DEVELOPMENT OF EIMERIA

The site specificity of Eimeria within the host is so well established that only a few species of the genus Eimeria are known to develop in sites other than the intestine. Eimeria truncata and E. stiedae develop in the kidney of the goose and the liver of the rabbit, respectively, while E. neitzi develops in the uterus of the impala. There have been other reports of a coccidium (probably a species of Eimeria) occurring in the placenta of the hippopotamus.

Studies on the site specificity of species of Eimeria parasitizing the chicken were made by Horton-Smith and Long (1965, 1966), and these indicated that for some species the site of parasitism was close to the point in the alimentary tract where sporozoites emerged from the oocysts. This suggested that selection is determined largely by the interplay of host and parasite factors which bring about excystation of the invasive stage (sporozoites). With E. tenella the sporozoites emerge from the oocysts in the small intestine, yet they initiate infection of the caeca less than 1.5 h after the introduction of the oocysts by crop intubation. Farr and Doran (1962) showed that oocysts of E. tenella, which parasitize the caeca, release the sporozoites appreciably slower than those of E. acervulina, which parasitize the duodenum. Similarly in the turkey the excystation time of E. gallopavonis from the caecum was longer than that of E. meleagrimitis from the duodenum. If the sporozoites are introduced intravenously, they initiate infection of the normal intestinal site (Sharma and Reid 1962, Davies and Joyner 1963, Long and Rose 1965). In another study, Leathem (1969) produced only minimal infection with E. tenella in the small intestine of caecectomized chickens, which suggests that sporozoites are normally attracted to the caeca by chemotaxis.

Parenteral inoculation of sporulated oocysts of E. falciformis

into mice resulted in the development of the parasites in the colon and caecum, the usual site for this species (Haberkorn 1970). Pellerdy (1969) intravenously inoculated rabbits with sporozites, oocysts and merozoites of T. stiedai and found development of the parasite in the usual liver site. However, Fitzgerald (1965) was unable to infect calves with E. bovis by the intraperitoneal route.

In chickens treated with the corticosteroid dexamethasone, schizogony of E. tenella occurred in the liver bile ducts and, in one case, in the midintestine (Long 1970a). No stages beyond the second generation schizonts were found and the parasites were surrounded by host defense cells. Chicken embryos have poorly developed immune mechanisms, and it is possible that successful infection of the embryo CAM by several species of Eimeria (Long 1965) may be due to this fact.

When embryos were inoculated intravenously with large numbers of cleaned sporozoites of E. tenella, schizogony occurred in the bile duct epithelium of the liver; no infection occurred at other sites. It is thought that they were removed from the circulation by the liver and consequently developed there; the liver of chickens appears to be highly efficient in the removal of foreign particles from the circulation (Dobson 1957).

Horton-Smith and Long (1965, 1966) examined six intestinal species from the fowl and showed that with three species the whole endogenous cycle could occur in the caeca if sporozoites were inoculated directly into this site. We have noted that those species of Eimeria which developed the whole of their endogenous cycle in the caeca were those in which development occurred to some extent in the caeca as a result of the usual (oral) route of infection. Schizogony of E. necatrix usually occurs in the small intestine, with gametogony occurring in the caeca. Horton-Smith and Long (1965) demonstrated that when sporozoites of this species were introduced directly into the caeca, the parasite completed the whole life cycle there. Similarly, E. brunetti and E. mivati, which normally parasitize posterior intestinal tissues, would readily develop in the caeca, whereas E. acervulina and E. maxima, which primarily invade the duodenum and anterior intestine, respectively, did not.

Eimeria praecox, a parasite of the duodenum, does not develop in the large intestine, and sporozoites obtained by in vitro excystation methods and introduced via the cloaca or inoculated directly into the caeca produced infection in the usual (duodenum) site (Long 1967). Recent work with E. praecox, E. maxima and E. acervulina showed that sporozoites of these species inoculated directly into the caeca fail to develop in this site. However, they do initiate infection of the small intestine with little or no delay in the normal prepatent time. Intracaecal inoculation might cause damage to the caecal wall so that sporozoites might "leak" into the peritoneal cavity and then

produce infection elsewhere. In order to overcome this possibility, sporozoites were introduced via the cloaca. Indeed, infections could be produced by placing sporozoites on the external area of the cloaca. It has been shown that retrograde movement of fluids to the large intestine and caeca occurs by this method (Akester et al. 1967). Infection of the usual (duodenum) site also occurred when sporozoites were introduced into the peritoneal cavity. These experiments suggest that the sporozoites migrate from the large intestine to the duodenum. In addition, E. praecox sporozoites were labelled with ^{51}Cr and introduced into the caecum or rectum, and counts above background were obtained 1-1 1/2 h later.

A number of species of Eimeria form gametocytes in sites different from those occupied by schizonts. In E. necatrix schizogony takes place in the intestine, and gametogony occurs in the caeca. Norton, Catchpole and Joyner (1979) found that with E. flavescens from the rabbit first-generation schizonts were formed deep in the glands of the lower small intestine and that the merozoites migrated to the caecum and colon where second, third and fourth generation schizonts develop in the superficial epithelium.

The results of infection by parenteral injection of oocysts or sporozoites suggest that either sporozoites or merozoites have appreciable powers of migration or that macrophages play a role in this migration. Parasites may be engulfed by macrophages and remain viable at least for limited periods (Huff and Clark 1970, El-Kasaby and Sykes 1973). After intraperitoneal or intravenous inoculation, trophozoites or early schizonts have been recovered from the liver (Long 1970a, Long and Millard 1976), and it is possible that they reach this site via macrophages.

Kogut and Patton (unpublished data) found that invasion of cultured cells by sporozoites of E. tenella was increased at atmospheres containing low oxygen. Little is known of the oxygen tension in the avian gut. Von Brand (1973) assumed that the intestinal environment offers little or no molecular oxygen. This assumption can be substantiated by the large numbers of obligate and facultative anaerobic bacteria found in the caecum of the chicken (Barnes 1972, Barnes and Impey 1970).

Thus it seems clear that special factors are involved in the habitat which attract the invasive stages of coccidia. These factors are decisive in determining the precise part of the intestine in which the parasite develops, with changes in the "normal" site occurring only when the host is greatly stressed or is highly incompetent immunologically. It seems likely that the determining factors in site specificity are likely to be related to local physiology of tissues which may or may not meet the requirements of a given species.

FACTORS AFFECTING THE CHOICE OF HOST CELL

With few exceptions, parasites of the genus Eimeria develop in epithelial cells, and most species develop in epithelial cells of the intestinal villi. Two of these exceptions (E. stiedae in the liver of the rabbit and E. truncata in the kidney of the goose) develop in epithelial cells of the bile ducts and kidney tubules, respectively. The species of Eimeria which are known to be parasitic in the chicken are found only in the epithelial cells of the intestinal villi.

In a number of studies (Doran 1966, Pattilo 1959, Van Doorninck and Becker 1957) it was suggested that sporozoites, after this initial invasion of the intestinal mucosa, were transported to the crypts of Lieberkühn by macrophages. These workers provided no definitive evidence that the cells harboring the sporozoites were indeed macrophages. They were identified as macrophages on the basis of their large, densely staining nuclei.

Millard and Lawn (1982) described the development of first generation schizonts of E. dispersa in intestinal interepithelial leucocytes. These cells are intraepithelial wandering cells which have been given a variety of names, e.g., epithelial lymphocytes, thelialymphocytes, interepithelial lymphocytes and granulated lymphocytes and may be related to globule leukocytes. Further support that the cells involved in sporozoite transport are not macrophages has been provided by Lee and Al-Izzi (1981). These workers treated chickens with carrageenan, known to selectively kill macrophages. Animals treated before parasite inoculation developed infections with E. tenella of similar severity despite severe effects on macrophage activity.

The first-generation schizonts of E. tenella and E. necatrix develop in the crypts of Lieberkühn, but the merozoites of these schizonts invade connective tissue cells between the glands and give rise to the large second-generation schizonts which are so characteristic of these species. The merozoites of this generation are thought to invade epithelial cells of the crypts and the superficial villi where gamonts are formed. The question arises as to whether these merozoites migrate away from the epithelium because of the unfavorable environment or whether they are carried away by host defense cells (macrophages). The grossly enlarged nuclei of the host cells harboring second-generation schizonts of E. tenella led Gresham and Cruickshank (1959) to conclude that development of this stage occurred in macrophages. It is possible that some of these stages do develop in macrophages, but it is equally possible that gross structural changes are brought about by the growth of these large schizonts within the cells and that this gives them the appearance of macrophages. This view was expressed by Long (1970d) and Fernando et al. (1974) as a result of studies

on E. tenella and E. necatrix. Bergmann (1970) studied the ultrastructure of cells from chicken caecal tissues infected with second-generation schizonts of E. tenella and concluded that growth of the parasites brought about changes in the host cells giving them the appearance of macrophages. Stockdale and Fernando (1975) and Fernando et al. (1974) found that parasitized host cells have an increased DNA content with an enlarged nucleus. They noted that the cells were joined by desmosomes and concluded that they were most likely to be epithelial cells. Fernando et al. (1974), working with E. necatrix, have shown that the host cells undergo extensive hypertrophy with at least a four-fold increase in the DNA and RNA content of the nucleus. However, the development of gametocytes of E. maxima caused extensive enlargement of the host cells without nuclear hypertrophy or enhanced DNA synthesis.

Pasternak et al. (1977) studied E. zuernii from calves. The first-generation schizont stage occurred generally but not exclusively in non-proliferative cells. Nuclear hyperplasia occurred in a majority of infected cells, but there was not a proportional increase in DNA synthesis.

Long (1971) demonstrated that schizogony and gametogony of E. tenella occurred in the liver of chick embryos inoculated intravenously with sporozoites and that gametogony occurred more freely in embryos which had been treated with dexamethasone. These schizonts and gametocytes were found in epithelial cells of the bile duct, but many gametocytes were also found within cells which appeared to be hepatocytes. Because of the difficulty in identifying tissue cells and macrophages, this work has been extended to the ultrastructural level, as the infected cells can be characterized by their organelles. We have shown that second-generation merozoites of E. tenella will invade and develop into gametocytes in hepatocytes and that development of asexual stages occurs in fibrocytes of the portal tract and in the endothelial cells which line the sinusoids. Merozoites which were occasionally seen within cells resembling macrophages were always in varying stages of destruction. Schizonts developed in all of these different cells, except macrophages, but gametocytes were detected only in hepatocytes (Lee and Long 1972). Long and Rose (1975) have made a number of attempts to grow E. tenella in macrophages cultured in vitro. First-generation schizonts were grown in only two experiments and only very few schizonts were found.

Eimeria tenella may develop in a variety of host cells, and it is interesting to question why they are normally restricted to specific cells in the caeca of the fowl. As already mentioned, the sporozoites of E. tenella are capable of invading a wide variety of cells in vitro, but development of schizonts, although occurring in fibrocytes, occurs most freely in epithelial cells.

Our observation that second generation schizonts develop in cells lining the sinusoids of the liver of chicken embryos shows that they are capable of development in mesodermal cells. Similarly, the observation that gametocytes of this species develop in hepatocytes in embryos is consistent with the development of this stage in endodermal cells. This work suggests that unfavorable conditions for invasive stages are present in most situations. However, once sporozoites or merozoites become intracellular they have excellent opportunities for survival and replication.

FACTORS AFFECTING DEVELOPMENT OF *EIMERIA* IN THE INTESTINE

The host has complete control over the ability of the *Eimeria* sporozoites to invade intestinal cells. Successful excystation of sporozoites depends first upon suitable conditions in the stomach of mammals (or gizzard in birds) in bringing about changes in the oocyst membrane so that later treatment with trypsin (or chymotrypsin) and bile stimulates sporozoites to be freed from the oocyst and sporocyst.

The speed of these reactions determines where, in the intestine, the sporozoites will be able to penetrate. Thus if aged oocysts are ingested, fewer sporozoites will emerge, and these will take longer to be freed from oocysts (Long 1970b, Doran and Vetterling 1969). In the very young chicken, where digestive enzyme activity is low, sporozoites will not be available in the anterior regions of the intestine, and sporozoites of duodenal species of *Eimeria* are able to develop further down the intestine.

The caeca appear to have a minor role in digestion of food, but the mechanisms by which they fill and discharge have been widely studied. It has been found that the caeca fill at fairly regular intervals and that valve-like structures at the entrances to the tubes act as filters so that only fluids with small particles can gain entrance. The evacuation of caecal contents occurs as a result of contractions which originate at the base of the caecum and pass along the body of this organ. These contractions occur at fairly regular intervals and depend upon the degree of caecal distension. Coccidia inhabiting the caeca appear to have overcome the effects of caecal evacuation mainly because the invasive stages (sporozoites and merozoites) are within cells or they stay close to the mucosa. It is only when present in the caecal lumen that they are lost in the caecal evacuations. In a recent study Clarke (1980) found that caecal contents were never voided during the hours of darkness and were voided mostly in the afternoon and evening. Birds heavily infected with *E. tenella* discharged hemorrhagic caecal feces during both light and dark periods. Thereafter, no caecal feces were discharged for several days due to the formation of caecal cores. It would be interesting to know the periodicity of oocyst production of *E. tenella* in lightly infected animals

and to judge when in the day oocysts are actually discharged.

Another factor affecting the movement of coccidial stages within the large intestine is that material from the cloaca, including urine, can be drawn into the caeca as a result of antiperistaltic movements originating at the cloaca. These movements have the effect of transferring the merozoites from the large intestine to the caeca and enable the life cycle to be completed in another site. This could explain how in E. necatrix infection the merozoites formed in the small intestine are transferred to the caeca for gametogony to occur.

EPITHELIAL CELL REGENERATION

Pout (1967) suggested that life cycles of species of Eimeria are synchronized with the rate of epithelial turnover of their respective hosts, the rate of epithelial cell turnover and endogenous cycles of Eimeria being shorter or smaller as compared with those of larger animals (Leblond and Walker 1956). A study of the life cycle of a number of Eimeria affecting chickens confirms this view in that first-generation schizogony occurs in the crypts of Lieberkühn and that successive generations of smaller schizonts occur along the sides of the villi in the next 1-3 days, with gametocytes and oocysts being produced in cells at the tips of the villi. It therefore appears that the endogenous stages of Eimeria have adapted to develop at rates which fit in with epithelial cell turnover and that after initial invasion by sporozoites of epithelial cells of the crypts of Lieberkühn the developing stages find themselves moving to more superficial areas in the epithelial cell "shunt" towards the tips of the villi. As each schizont generation matures, the merozoites invade new cells adjacent to the old host cells so that by the fourth to sixth day (the time needed for most species of Eimeria affecting chickens to complete their endogenous cycle) gametocytes and oocysts are produced at the tips of the villi, and the oocysts are then released into the intestinal lumen. In the chicken, epithelial cells comprising the whole crypt of Lieberkühn are replaced in about two days (Imondi and Bird 1966), and the endogenous cycles of chicken coccidia, comprising several asexual generations with gametogony, are accordingly of short duration (4-6 days). Despite the rapid turnover rate of epithelial cells, there is still a considerable loss of endogenous stages because many developing stages reach the tips of villi too early and are shed into the intestinal lumen.

Schizonts of some species (E. acervulina, E. praecox) take only 6-12 hours to reach maturity, but gut epithelial cells must surely divide every few hours. It would seem to be disadvantageous, from the parasite's standpoint, to develop within dividing cells, and observation of the intracellular stages suggests this is not a common phenomenon. However, it may be that Eimeria parasites are capable of slowing down epithelial cell

turnover to suit their own needs. Long and Millard (1968) showed that sporozoites of E. acervulina and E. tenella within cells of the crypts of Lieberkühn of chickens treated with an anticoccidial drug could survive for at least 60 days. The coccidia may inhibit the division of the progenitor cells which they invade or affect the rate of division of cells in the crypt as a whole.

CONCLUSIONS

A particularly interesting aspect in the biology of the host-parasite relations of Eimeria is the site specificity shown by the different species. Is this apparent selection determined by the parasite, or is it brought about mainly by host mechanisms acting upon the parasite? That is, does the parasite choose the most suitable from a range of possible sites, or is it forced, by the host, to occupy one site only, all others being unsuitable for invasion and development?

Research seems to show that many intestinal species of Eimeria develop in sites where sporozoites emerge from the oocysts, while other species must migrate or be carried to other sites before they can develop. Thus it seems clear that some species are, to some degree, flexible in their site for development in the intestine but that other species show a marked degree of site specificity.

The results so far discussed indicate that Eimeria parasites are capable of developing in a wide range of host cells in vitro. Carefully conducted in vivo experiments suggest that at least with some species the site for development is determined by host factors.

When immune mechanisms are diminished by treatment with immunodepressants (betamethasone, dexamethasone, cyclophosphamide), site specificity can be affected (Long 1970a). This work indicates that the strong site specificity of these parasites is most probably determined by the host and not the parasite.

Asexual stages of Toxoplasma gondii develop in a wide range of cells and sites in a number of hosts. There are only a few exceptions to the general rule that species of Eimeria develop in epithelial cells within specific sites. Gametocytes and oocysts of Eimeria are usually found in epithelial cells, and although Toxoplasma develops in a wide range of cells, gametocytes appear to develop only in epithelial cells of the cat intestine.

Having found a suitable site (and suitable cells within this site), the sporozoites must penetrate the cell; sporozoites may enter and leave cells of various types before they settle in a suitable cell. Eimeria appear not to be destroyed by host cells, as they are capable of leaving and entering other cells.

The parasites appear to be able to arrest the division of the host cell, with resultant hypertrophy of the host cell nucleus and enhanced nucleic acid synthesis. These changes are probably necessary in order to sustain the nucleic acid requirements of Eimeria during the rapid nuclear divisions leading up to the formation of mature schizonts and microgametes. Most merozoites appear to escape from schizonts and manage to avoid being destroyed by host defense mechanisms by rapidly invading new epithelial cells.

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DISCUSSION

BOTTJER: Would you care to speculate on how the sporozoites move around, or are they being moved by the cells?

LONG: Well, I am sure they are moving actively. In order for them to enter a cell, I assume that they must be pushing against something at their rear, but we can't see that happening.

FAYER: Under the membrane surrounding the parasite are approximately 20 to 25 longitudinal microtubules that extend along the length of the parasite, and they form sort of a skeleton to shape or change the shape of the organism so that it can move in various ways.

LONG: In a cell-free environment, all I ever see them doing is gliding or flexing. When they are near a cell, they seem to increase their activity. I don't think that we see all that's happening, but it is difficult to imagine a sporozoite's going through a cell without being able to push against something solid posterior to it.

FAYER: Once they are intracellular and round up before becoming schizonts, parasites lose the subpelecular fibrils and microtubules. However, they still can leave the cells.

CURRENT: Because of the way they are arranged, I believe these subpelecular microtubules may have some actin-like proteins associated with them and the plasma membrane. It could be a mechanism similar to that we've seen in some of the flagellates in which actin-like elements are attached to the cytostome membrane and associated microtubules. Using the subpelecular microtubules as a skeleton component, the contractile proteins actually pull the membrane during food vacuole formation. It is speculative, but there's a possibility that the parasite membrane is in a similar manner producing motility as it rolls out the merozoite or sporozoite.

FAYER: The rhoptries at the anterior end of the sporozoite are thought to release polyhistidine-like chemicals that influence the host cell membrane, allowing the organism to enter. This is strictly speculative. We really don't know how sporozoites move or how they actually enter cells.

BENNETT: When highly charged amino acids such as basic proteins are released, they do permanent damage to the membrane. If that were the case, you would expect that there would be some damage. In the film it appeared to me that there was no significant damage to the membrane once the parasite entered the cell. It almost appears that the sporozoite is releasing some kind of material that causes a phase transition in the lipomatrix and allows the parasite to then pass freely, or more

rapidly, into the intracellular compartment without creating damage.

LONG: There's been a lot of argument on this question. Some workers describe a double wall and not a single wall forming the host cell membrane. All it needs to do is get inside the first membrane and then this other membrane will just simply stretch around it. Eventually the whole parasite is inside the stretched membrane. The space enclosing the parasite becomes the well-known parasitophorous vacuole. And then I suppose if you say the parasite is inside the parasitophorous vacuole and the parasitophorous membrane is bounded by host cell membrane, one could postulate that it's not an intracellular parasite at all, because it doesn't have direct contact with the host cell cytoplasm. This would be a very arguable point.

FAYER: That's hypothetical also. There are several suggestions on this point. One is that the organism as it approaches the cells releases something so that the cell membrane is pushed in as you would push your finger into a balloon, and then that membrane pinches off behind the organism. As Dr. Long has shown, you have an organism surrounded by part of the host cell membrane within the cell. Another possibility is that a hole is actually poked in the membrane and the organism enters the cytoplasm. Subsequently, a membrane is formed de novo around that organism. So we are really not sure exactly what's happening. And it may differ with different species of Eimeria.

ERNST: Jim Jensen, working with Allen Edgar, did some very elegant experiments in which he studied entry of sporozoites in cell cultures by light microscopy. He also sectioned the penetrated cells using electronmicroscopy and Ruthenium Red. His work showed that the sporozoites did not disrupt the membrane.

EDGAR: I think our pictures led us to believe that the membrane was around it and it was pushed in, but there's still some argument about this. The thing that intrigued me about the sporozoites as I watched them by the hour in a tissue culture was that they looked like they might be making use of objects to move about. In clear fluid without any cells at all, they will show one little gliding motion, which makes me believe they make use of the musculature to propel themselves in fluid without any cells.

LONG: Yes, but they are not free in fluids. They are all resting on the bottom of the slide on a solid surface.

EDGAR: Well, they could be. In your pictures you showed shots where it looked like the sporozoites were either on top or below the cell. And some of them looked like they go right straight through. Jim and I used to argue when we watched

this. How would they make a hole in there without breaking any of the cells? They would penetrate and then come out the other side.

LONG: In some papers on this subject, the pictures show that in some species of Eimeria there may be some sort of a hole. In other papers, the pictures show the entry of sporozoites to be in a way similar to that you have been talking about.

EDGAR: I came to the conclusion that maybe both mechanisms occur.

ERNST: C. A. Speer has some other films in which the membrane is disrupted sometimes and the cell loses its contents. So sometimes there is damage, but most of the time there is not.

FAYER: I can add to that. You never see damage when these sporozoites enter the cell like we see cytoplasm leaking out when the sporozoite leaves the cell.

LONG: I can't really believe that in vivo the parasites would be entering and leaving the cell all the time. I think it's just because this is a false situation of kidney cells and that if you studied entry of true target cells, you would find the parasite would not bother to come out. Is it doing all this because it's really not in the right place? If you believe that they would try to penetrate any sort of cell, you would have to postulate that in Eimeria tenella infection penetration occurs all the way down the intestine, with the parasite entering and leaving cells until it reaches the large intestine. Dr. Hammond suggested this to me. My argument has always been that when we inoculate animals by mouth with E. tenella oocysts we can find sporozoites penetrating cells 45 minutes after inoculation. Now, if they were going in and out of cells all the way down the small intestine, then they would never reach the caeca in such a short time without running out of energy.

BENNETT: After hearing the comments that have been made about the higher probability that the membrane of the host is being capsulated from the parasite, it appears to me that when the parasite comes into contact with the host cell, there is an immediate trigger mechanism that seems to set off the process that we all know as endocytosis. That is, the object on the outside has struck the surface and has elicited a chemical event which the surface recognizes, and the cell immediately engulfs the parasite. All cells are capable of doing that. The question is, "What's the trick that some cells have and other cells do not?" And you wouldn't think the kidney cells, for instance, would be capable of endocytosis. But we really don't know if that kind of biochemical mechanism really exists in all cells. Perhaps there are some common mechanisms which, operating in conjunction with chemical substances on the tip of the sporozoite, might play a role in parasite invasion.

LONG: Dr. Fayer observed that quinine sulfate blocked invasion. I don't know what mechanism that was.

FAYER: When you add the quinine to cell cultures with sporozoites, the sporozoites do not penetrate. They reach the cell surface, but they simply will not enter. You take the same cells and the same sporozoites and wash the quinine out, and they will enter. What the quinine is doing, I don't know. it may be blocking receptor cells. But you do not get penetration of malaria, Toxoplasma, Besnoitia, or Sarcocystis in the presence of quinine.

LONG: I really can't see from all that has been done that we have any basis for the idea that receptor sites are involved in the invasion. If we compare Eimeria with Plasmodium, where receptor sites on the red cell surface are proven, Eimeria seems to differ. Why should a kidney cell have a receptor site for Eimeria, when the Eimeria parasitizes cells in the intestine?

DESPOMMIER: How do you interpret the probing action, and if it isn't looking for a site or receptor, what is this behavior pattern involved with? All the membranes should be about the same.

LONG: The conoid and the rhoptries are probably involved and essential for cell penetration. It may be necessary for sporozoites to make the breach at a weak point.

DESPOMMIER: Is it tasting or feeling?

BOTTJER: If it is a protein as you say, then it may be tasting.

LONG: Well, that's what it looks like.

BOTTJER: Then it may be secreting substances from the rhoptries which trigger the transition of the membrane to a liquid state, allowing it to move through. Does anybody really know? Has anybody isolated rhoptries in large numbers or been able to tell what's inside the rhoptries?

FAYER: Maybe I can reply to that. We've done some high-speed cinematography on penetration several years ago and found that penetration of the membrane from the time the sporozoite approaches the membrane until the time it is through the membrane takes place in less than a tenth of a second. So I don't know how long all this methylation business takes. But it doesn't take the organism very long to go through; it's quite rapid.

CASTRO: You have got to think of a relatively fast mechanism for changing the membrane structure. There aren't too many available.

BENNETT: A tenth of a second reminds me of that cytoskeletal membrane. As soon as that thing comes into the right contact, it pulls that membrane back and brings the parasite in with it. And it doesn't necessarily have to be a receptor mediator. It could be something initiated purely by the parasite.

LONG: Well, there are two things I can think of which would support your idea. One is that we do see multiple infection of the same cell, and most of us are a bit surprised by this. There are some remarkable pictures of sporozoites all lined up facing the same direction inside the cells with lots of cells all around not being parasitized. With Toxoplasma we have the description of penetration-enhancing factor (PEF) which collects the supernatant cell cultures used for growth of Toxoplasma. When PEF is added to another culture, greatly enhanced penetration of Toxoplasma occurs.

FAYER: I believe the PEF concept is wrong, because the investigators who described it have stopped promoting it. We have been unable to find such enhanced penetration with the Eimeria.

EDGAR: I'd like to go back and take up the host side. I think we need more chemical studies done. When we consider different genetic background of chickens, about 25 years ago we developed what we called R (resistant) and S (susceptible) lines. After you got about six or eight generations, you're about as far apart as you're going to get. Even 500,000 oocysts didn't kill very many. We had a coccidium parasitizing the lower small intestine and the caecal pouches. Instead of being in the central portion of the caecum, it was in the proximal portion. I think we should take the two lines and see what happened in vitro. We still have the line. With studies on a resistant inbred line in Wisconsin, E. tenella was never seen outside the caecal pouches.

LONG: I didn't see changes in the site of E. tenella in partially immune chickens.

EDGAR: These were susceptible chickens.

BONE: If you take an immune or partially immune bird, do you get any change in the sites?

LONG: In birds partially resistant to E. tenella, the light infection occurs in the caeca only. Furthermore, the small population which develops in immune birds does so right on schedule. There's no difference in prepatent time.

CURRENT: In any of your chicken systems, do you see parasitism of particular villi in the intestines; do you see colonization of certain villi? You say that in the cell culture you may find one or two cells where you find a lot of parasites penetrating one or two cells and a whole array of cells around

without parasites? In our Isospora in the swine, we may find a hundred villi that are not infected and then there will be one villus where just about every cell is infected.

LONG: Yes. This is a colony. You may see the whole of one villus infected with almost every host cell infected. In the same area, there are adjacent villi perhaps not infected at all. I think that's a different explanation. I think all it really means is that once the sporozoite has invaded a cell, the merozoites that are released from various generations of schizonts don't migrate very far, if at all. I think they are just going into the cell right next to them. So you get villi which are heavily parasitized with adjacent ones which are not.

CURRENT: I have a couple of comments especially related to this cell that picks up the sporozoites or carries it. We have never seen anything in our work that would lead us to believe such a thing happens. Is it only E. tenella, or does this occur with all the Eimeria of the chicken?

LONG: I think it's all the species. Doran described sporozoites being transported in macrophages in the chicken. Sporozoites do not appear to go directly to the crypts.

CURRENT: What you're saying is these parasites are crypt parasites to begin with.

LONG: Yes, almost all of them that I have studied.

CURRENT: How about those that are not crypt parasites?

LONG: E. praecox is the only one I know of where parasitism of the crypts does not seem to occur.

ERNST: You're saying the chicken species originate in crypts? We don't get too many like that. A lot of our rodent species start up the sides of villi and complete development at the tips of the villi. The development time of the parasite is related to the length of the life of the epithelial cell. I certainly cannot agree with this for infections in mammals or in rodents. We get sporozoites and other stages that are right at the tips of villi. And this is where these epithelial cells are going to slough off. Or we'll get some species that are on the sides of the villi for several days. And this would lead you to believe that the longevity of the host cell is extended. This was brought up at a symposium in Beltsville a few years ago where the longevity of epithelial cells and how the parasites manage to develop were discussed. Nobody really knows how long these epithelial cells live.

LONG: I think they know what the longevity is. I'm not sure that they know what it is once the parasite is intracellular. The parasite must slow down host cell division. The metabolism

of the host cell is affected. It grows very large; it must do so because the growing parasite is bigger than the host cell anyway.

FRANDSEN: That's certainly true in the rat. In the literature it is known that the turnover rate in the rat is very rapid and the parasite could not develop at that same rate.

LONG: There are several possibilities. One is that parasitism actually slows down cell turnover rate. Alternatively, when the parasite chooses another cell to invade, it goes back down towards the crypts rather than superficially.

DESPOMMIER: The problem is true for Trichinella also. So it's not just one cell. It's about 400 cells. It's always in the same place when we look at it in sections, and yet we know that the turnover rates are very rapid.

CASTRO: Cell turnover is probably slowing down in the case of coccidia and possibly Trichinella. As far as I know, there haven't been too many measurements of cell-turnover time. One of them has been made by Lloyd Simon. He showed an increase in the turnover rate. That was the situation where you have a lot of inflammation which occurs in the Trichinella, but there is not so much with coccidia. In the cell-turnover count you have to be cautious about making general statements about parasitism depending on whether the parasitized epithelial cell divides.

LONG: I think they did find that, strangely enough, it speeded up. I think what we're talking about now is whether the parasitized cell is in a different situation from the non-parasitized cell, because the minimum time it needs is 12 to 18 hours to complete its development. This is much longer than 20 minutes or so needed for epithelial cell division.

FAYER: There are some notable exceptions. For example, in Eimeria bovis sporozoites will enter a cell, and it will sit there about 12 or 13 days without really much observable change.

LONG: It's not in an epithelial cell, though. It's in an endothelial cell.

EDGAR: In sheep, some species also have large schizonts. None of these occur in the epithelial cell of the gut. They occur in the lamina propria or in the lacteals. None of the very slow-growing ones that I know of in the mammals occur in the epithelial cells. Later generations do.

CASTRO: I'm still not clear on these intercellular leucocytes in terms of the life cycle. Are you saying they have to be there to initiate the life cycle, and are these cells the ones which are first penetrated by the sporozoites?

LONG: Not all the species have been examined yet. But in E. tenella in the chicken, it appears to be the first cell that the parasite gets in. I showed that in E. dispersa in the turkey, it is undoubtedly the first cell penetrated.

CASTRO: Somebody mentioned E. nieschulzi, and there was some work I think that was done by Elaine Rose in an athymic rat where the cycle just kept going on and on. The animal never did develop immunity. And there's also some work by Ruitenberg with athymic animals, primarily the mouse. I'm not sure about the rat. She said she thinks that the globule leucocytes are derived from mast cells which are very deficient in the mouse. If we can extrapolate from that to the rat, then we have a rat without globule leucocytes; yet we can continue to get this reinfection in these animals.

LONG: Yes. I don't know whether in the rat coccidia these cells are essential for the first part of the life cycle.

FAYER: You are talking about the globule leucocytes, are you not?

LONG: This is a problem. The cells I mention don't have globules in them. The interepithelial leucocytes are different cells from globule leucocytes.

CASTRO: OK. Maybe there are a lot of cells that can be in there, but you're saying yours is a lymphoid-derived cell?

LONG: We believe so.

CASTRO: Ruitenberg is saying it's a modified mast cell. But then the mast cell in turn is lymphoid-derived. We are probably talking about the same type of cell.

FAYER: That doesn't look like a globule leucocyte to me.

LONG: I can show you lots of pictures of globule leucocytes. I've never seen a parasite inside a globule leucocyte. And we get masses of them in animals which have recovered from coccidiosis. We did a lot of counts on them because we thought they were very important.

CASTRO: What are you calling this cell? I thought you gave us several synonyms for it?

LONG: Well, yes. Some people think globule leucocytes are related to the cells--I don't. Whether it's an immature form of cell, I don't know.

CURRENT: These lymphoid-derived transport cells that you're talking about, do you see an increase in the number of these cells in an infected versus non-infected animal?

LONG: I can't answer that because the only way that you could really be sure of identifying them is by EM work. I think all that work is going on, but I think it's too early to say.

CURRENT: What I'm wondering is, are these cells present in normal animals?

LONG: Oh, yes, I'm sure of that.

CURRENT: And in numbers sufficient to transport that many sporozoites to the crypts?

LONG: Yes. I should think so in the material I have looked at so far.

EDGAR: Where do you think the lymphoid transport cells pick up the sporozoites, and where do they go? They must go into the crypt.

LONG: Oh, I think they're right in between the surface epithelial cells. Actually superficial to the host cell nucleus. It's a long transport--not a very short transport. I think they are getting between these cells within a few hours of penetrating.

EDGAR: Where? Up on the villi or down between the villi?

LONG: I think they are transported via the lamina propria. Most of the sporozoites I showed you are coming in here. The transport cells are actually between cells, not intracellular. They travel into the crypts. Once in the crypts they leave these cells and enter epithelial cells to develop.

EDGAR: What kind of cell do you think harbors the second-generation schizont of E. tenella?

LONG: We formerly thought that the second-generation schizonts were always in the submucosa, which led one to believe that they developed in subepithelial cells. But what I'm saying now is that the parasites are so enormous they have actually completely destroyed the crypt they are in. I now believe they develop in epithelial cells.

EDGAR: If you look at these cells carefully, you will always see a large nucleus, which always makes me believe they are some type of epithelial cell.

LONG: I think it's an epithelial cell. Although they look huge at times. I think in order to sustain growth of the enormous schizonts (between 50 and 80 microns in diameter), which take two days or more to develop, the host-cell division must shut off. The host cell has tremendous RNA and DNA synthesis. These have been measured and they contain an enormous

amount of RNA and DNA--just to sustain the growth parasite, I think.

EDGAR: That doesn't bother me at all. The only part that bothers me is the cell supporting the first generation schizont.

LONG: They are in the crypts and within the epithelial cells.

KLESIUS: Since the genetics of the host has been brought up, I might say that we recently finished some work indicating that the acquisition of resistance is definitely under genetic control. And it's a dominant trait. One other comment about the intercellular or epithelial leucocytes. They don't undergo blastogenesis. So they're probably not just one lymphocyte type. In other words, you might have subsets which are known to occur in these cells.

BONE: Actually they can be. That is something we might have a go at. It would be quite interesting.

THE INFLUENCE OF HOST IMMUNE RESPONSES ON PARASITE BEHAVIOR

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INTRODUCTION

My subject is a highly speculative one. As we shall see, there is little hard evidence to support the idea that host immune responses can affect parasite behavior. On the other hand, the available evidence does suggest that the phenomenon exists and, moreover, that further investigations in this area are warranted.

Some ground rules proved helpful in the development of this topic. I will follow Whitfield's (1979) lead when, in discussing the behavioral aspects of symbiotic (sensu latu) associations, he restricted himself to metazoan animals with nervous systems. Although this position is certainly arguable, I found it convenient, both from the point of view of definitions of behavior and with respect to my own area of familiarity. Regarding the former, Whitfield stated that "Purely by convention, the behavioural dimension becomes less well defined as the organisms involved become simpler. The responses of bacteriophages to host bacterial cells, or of algae to fungi in a lichen, are behavioural in a sense, but are more usually described in the language of biochemical, cellular or physiological events." Of course, persons studying metazoan parasite behavior are reductionists, too; so, in those few instances where such lower-order events could be implicated, I found no hesitancy in discussing them. With respect to my own perspective, I hope that I will be forgiven if I draw examples mainly from the research on endoparasitic helminths.

Whitfield's treatment of symbiont behavior was also useful to me in that he distinguished "association-specific behaviour" from behavior common to symbionts and their free-living relatives. Furthermore, he provided a helpful categorization of association-specific behavior, around which I will build my own discussion. Whitfield's categories are: (a) associate finding, (b) associate recognition, (c) establishment behavior,

and (d) maintenance behavior. For my purposes, I find it useful to include a fifth category of association-specific behavior, which I will call "propagative behavior."

SOME POSSIBLE EXAMPLES OF IMMUNE RESPONSE MODULATION OF PARASITE BEHAVIOR

The immune system can be implicated if a parasite's pattern of behavior is altered in immune, immunodepressed, or passively or adoptively transferred hosts. Proofs of immune system involvement require deeper investigation. The examples reviewed below are, at the least, almost purely speculative and, at the most, only suggestive of immune system involvement, by the above criteria.

Associate Finding

This category of behavior occurs in those parasites possessing free-living dispersal stages (the active host-finding category of MacInnis [1976]). Whitfield (1979) considers this to involve chiefly responses to signals from the environment. Environmental signals may also be altered by potential hosts, as was demonstrated for snails and miracidia (Sponholtz and Short 1976). Since non-specific phenomena occurring outside the body of the host are involved in associate-finding, it is difficult to imagine how the immune system could influence this category of behavior, other than in the indirect way that dispersal stages produced in immune hosts could be less vigorous, hence compromised, in their abilities to survive and respond to the appropriate cues. I know of no evidence to support this, however.

Associate Recognition

In this category, the dispersal stages perceive and respond to specific stimuli emanating from the host. Again, I am unaware of evidence to support an influence of immunity on such processes and can speculate on only two ways in which it might come about:

1. The immune state itself could result in altered release of specific chemoattractants into the environment.

2. In the special case of fish, it is known that antibodies are secreted into the surface mucus (Fletcher and Grant 1969, Bradshaw et al. 1971). Though unlikely, it is conceivable that their presence could modify host recognition in either specific or non-specific ways.

Establishment Behavior

After finding and recognizing a potential host, the parasite must establish itself on or in it. This requires either

attachment or penetration (even in those parasites that are ingested). Although interesting possibilities exist with respect to immune intervention with attachment by ectoparasites such as ticks (Trager 1939, Wikel and Allen 1977), I will dwell instead on endoparasitic helminths. Many of these have larval stages that actively penetrate skin (e.g., schistosomes, hookworms, Nippostrongylus), mucosal surfaces (e.g., Ascaris, Nematospiroides) and other tissues (e.g., muscle cells by Trichinella spiralis).

Evidence can be found to show that the success of penetration is lower in immune as compared to naive hosts. Whether any of this can be attributed to effects on parasite behavior is questionable. Here I wish only to list a few examples of an immune effect on establishment and will discuss their behavioral implications later.

In schistosomiasis research, a "gatekeeper" role for the immune response has long been an attractive hypothesis. It received impetus with the report of Ogilvie et al. (1966) that homocytotropic antibodies in the sera of Schistosoma mansoni-infected rats could, upon passive transfer into the skin of recipient rats, protect against a percutaneous challenge, through the skin injection site, of cercariae. Their experiments, however, allowed no conclusion to be drawn regarding the actual site of parasite loss. Only much later was augmented skin loss of penetrating schistosomula (in immune mice) actually demonstrated (Smithers and Gammage 1980).

Parenteral immunity in Trichinella spiralis infections in rats (Ruitenberg and Steerenberg 1976) and mice (James et al. 1977) may be another example of an anti-establishment effect. The exact nature of the vulnerability of the new-born larvae is unknown. Although a stage-specific in vitro killing mechanism, involving antibodies and eosinophils has been described (Kazura and Grove 1978), we do not know if this is effective in vivo and if other processes serving to curtail establishment, for example, interference with muscle penetration, also operate.

Anti-establishment effects of the immune response have long been known for larval cestode infections. Early work showed the efficacy of immune serum passive transfer against establishment by larval taeniids (Campbell 1938, Leonard and Leonard 1941). Subsequently, protection was shown to reside in the IgG_{2a} antibody fraction in rats infected with Taenia taeniaeformis (Leid and Williams 1974). Possible involvement of the immune response in preventing establishment of Multiceps serialis larvae in mice is suggested by the work of Esch (1964) who found that cortisone treatment increased the infection rate in mice fed eggs of the tapeworm.

The examples dealt with above, as mentioned, are probably not attributable to immune effects on behavior. More likely, they

represent immune-mediated death in penetration or post-penetration sites. But what of invading parasites which manage to escape the "gatekeeper" mechanisms? Is there any evidence to suggest that their subsequent behavior is altered in the immune host? For two skin-penetrating helminths at least, Nippostrongylus brasiliensis and Schistosoma mansoni, there is good evidence of a migration delay in immune hosts (Love et al. 1974, Doenhoff et al. 1978). The former paper is additionally instructive because it clearly demonstrated the existence, in the case of N. brasiliensis in the rat, of a marked, local gut level barrier to the establishment of esophageal L_4 larvae. But in answer to the questions raised above, the authors also found that swallowed L_4 larvae--the survivors of the initial immune pre-lung destruction--had not been harmed by their sojourn in an immune host. But their behavior was changed, in that their migration had been delayed. The oft-noted immunity-associated delay in migration of schistosomula is probably related to both host and parasite factors. The knowledge that the entire migratory phase is intravascular, involving multiple circuits in the pulmonary-systemic circulation (Miller and Wilson 1980) suggests several interesting possibilities. Miller and Wilson thought that the normal stimulus for sequestration in the hepatic portal system might be the high levels of nutrients found there. If migration delay involves longer recirculation, then either immunopathologically-induced changes in vascular architecture (Dean et al. 1981) or interference with normal stimuli for migration termination (Dean, D.A., personal communication) could account for the delay. The latter would constitute a very interesting example of an immune effect on parasite behavior.

Maintenance Behavior

This phase of behavior involves the long-term relationship between parasite and host. One obvious behavioral characteristic that has been related to host immunity is change in location in the host. For example, Panter (1969) found that the distribution of both larvae and adults of Nematospiroides dubius was altered in the small intestines of immune as compared with normal mice. A differential effect of the host immune response on site selection in the guinea-pig intestine by adults and third-stage larvae of Trichostrongylus colubriformis was noted by Connan (1966). Adult worms began a posteriad migration in the gut of immune guinea-pigs several days before a second generation of worms began their posteriad migration. Neither stage migrated in irradiated hosts, suggesting but not proving immune system involvement.

Immune response-induced changes in site preference among lumen-dwelling intestinal helminths are not universal, however. For example, Kennedy (1980a) showed that even in mice primed for expulsion of Trichinella spiralis, any portion of the small intestine provided an adequate environment in which the

(shortened) life cycle could be completed and that the parasites did not migrate in immune hosts.

Propagative Behavior

All parasites must propagate themselves. Most of the associated patterns of behavior, copulatory behavior for example, are broadly shared with free-living relatives and so would fall into Whitfield's category of behavior which is not association specific. However, there are some aspects of behavior in propagative functions that are clearly related to the nature of the association itself, and therefore justify inclusion in the present category. Among these would be movements or migrations related to oviposition, such as the well-known nocturnal, perianal peregrinations of female pinworms and the ingression of the female schistosome into small venules (Fairley 1920). The periodicity of microfilariae in the peripheral circulation could also be considered an example of association-specific propagative behavior.

A change in propagative behavior of *S. mansoni* occurs in certain experimental and human infections. This is an anterior shift in choice of oviposition site along the intestine, which was variously associated with duration of infection (Cheever and Powers 1969), immunity (Foster and Broomfield 1971), or presence of Symmer's fibrosis of the liver (Cheever 1968). The immune response has been suggested to be at the basis of this movement of the adult worms (Cheever and Powers 1969).

Non-association-specific Behavior

The pairing of dioecious and in some cases monoecious (e.g., *Paragonimus westermani*) parasites in their hosts represents an aspect of their biology that may well be susceptible to immune interference or modification. Yet, this area has been virtually unresearched, and I am unaware of any reports which directly bear upon it.

SOME FURTHER SPECULATIONS

The evidence reviewed above, although meager, does suggest that a concerted effort to delineate immune-response modulation of parasite behavior could yield interesting and potentially valuable information. It may therefore be of some use at this time to suggest some ways in which immune responses could be both perceived and ways in which they could act to modify parasite behavior.

Perception of an Immune Response

Direct perception. Ordinary sense organs subserving the functions of mechano-, chemo-, and rheoreception probably do not provide direct sensory input of an immune response. However,

information regarding a surface immunological reaction in parasites bounded by a cell membrane or tegument may be gained by receptor-ligand binding and subsequent cytoskeletal disturbances by transmembranal signals. The antigen modulation by adult schistosomes, recently described by Kemp et al. (1980), is an example of a phenomenon that may well fit this scheme and that may confer a measure of survivability to the parasite.

Indirect perception. It is easier to imagine indirect mechanisms whereby an immune response could be perceived by a parasitic helminth. This could occur by immune interference with normal perceptions. In these cases, immune effectors would block or otherwise interfere with mostly chemoreceptive functions. This could come about in several ways: (1) chemoreceptors could be blocked by steric hindrance or more specific binding of antibodies, or (2) gradients of chemoattractants (including pheromones) could be altered. With respect to the latter, if the chemoattractant molecules themselves are antigenic, they could be complexed by antibodies. Another possible mechanism by which chemical gradients could be altered is by local inflammation, and attendant cell secretion of pharmacological agents or mediators which could augment, bind, or degrade those very molecules to which the parasite responds. An example here would be the release of 5-hydroxytryptamine by both vagal stimulation and from mast cells by anaphylaxis in the rat, and the postulated role of vagal stimulation of gastrointestinal function in the intestinal migration of Hymenolepis diminuta (Mettrick and Cho, 1981).

Mechanisms of Action

It is the thesis of this discourse that the occurrence of an immune response, whether or not directly perceived by a parasite, can result in changes in the parasite's behavior. Possible examples were reviewed above. The purpose of the following discussion is to sketch some scenarios in which the links between immune response and behavior might be traced. I admit to free exercise of my imagination, as well as extensive borrowing from the thoughts of others, and only hope that the effort serves as a fillip to research.

Direct mechanisms. Immune-induced stress could directly alter parasite behavior. The tegumental antigen modulation in schistosomes mentioned above, could, even while protecting against immune attack, at the same time be stressing the worm since Kemp et al. (1980) have shown the phenomenon to be dependent upon glycolysis. Another possible related example is the modulation of nematode acetylcholinesterase isozymes by antibodies (Jones and Ogilvie 1972). Such immune-induced demands on biosynthesis should be capable of altering parasite behavior, particularly that related to feeding.

The following hypothesis, which I have developed to explain reduced fecundity in schistosomiasis, can serve as a more detailed illustration of the way in which the immune system might act to result in a modification of the schistosome's propagative behavior, namely the choice of oviposition site.

Reduced fecundity, dissociable from adult worm death, is now recognized to occur commonly in various experimental schistosome infections. The coincident phenomena of anterior shift and reduced fecundity in baboon schistosomiasis mansoni were linked by Damian et al. (1976) as follows: We suggested that the suppression of egg production which we observed in chronically infected baboons was a result of the host immune response. Reduced fecundity could have resulted from slow starvation of the schistosomes in an immune host. In this scheme, the adult worms, "confronted with immune interference to adsorption of glucose or other essential nutrients, might respond by migrating to the nutritionally richer environment of the small intestinal mesenteric veins from the colonic vessels." Furthermore, we favor the hypothesis that the immune effectors responsible for starvation are antibodies directed to the tegumental surface, even though the same end could conceivably result from the absorption of "heterospecific" antibodies, as documented by Kemp et al. (1977), if steric hindrance of transport sites is a consequence of such immunoglobulin absorption. In this case, however, the effect of immunity would be nonspecific, depending only upon an elevation of plasma immunoglobulin levels, as is known to occur in schistosomiasis (Antunes et al. 1971, Cook et al. 1972).

It must be reiterated that the above scenario is but our current working hypothesis, and much work needs to be done to establish or refute the postulated links between immunity, nutrition, fecundity, and behavior. Yet, in other parasitic helminths, namely Nippostrongylus brasiliensis and Nematodirus battus, there is excellent evidence to support the existence of links between immunity and parasite energy status. Ballantyne et al. (1978) found in both of these nematodes that their adenylate energy charge values fell during the course of infection, while immunity was developing. These authors also forged conceptual links between immunity, nutrition, fecundity, and behavior.

Another example of how an immune response might alter parasite behavior was provided by Lee (1969). He speculated that, in the case of Nippostrongylus brasiliensis, the immune response could affect normal thigmotactic responses. Noting that adult worms aggregate in vitro, Lee suggested that thigmokinesis would tend to keep the worms in the intervillar spaces of the intestinal mucosa. If the immune response could in some way "over-ride this thigmokinetic behaviour of the nematode," it would "drive the nematodes away from the mucosa," causing the parasites to move about in the intestine in search of a better

environment. Unfortunately, no suggestions as to how the immune response might affect thigmokinesis were offered.

These two examples should suffice to illustrate at least the forms and complexity that mechanisms directly linking the immune response to parasite behavior might be expected to have. Still to be considered are possible indirect mechanisms.

Indirect mechanisms. Two possibilities for indirect immune intervention with parasite behavior come to mind. The first is immune response modification of parasite environment, with subsequent changes in parasite behavior. This is not strictly separable from some of the ideas considered above, for example serotonin release by both immune and non-immune triggers, but might be reserved for those aspects of environmental modification resulting from non-specific consequences of specific immune reactions. These possibilities have been thoroughly discussed by Castro (1976), so will not be further considered here.

The second indirect linkage could occur as a result of an immune response acting upon a co-existing, possibly competing, parasite, with either release of competition pressure leading to behavioral changes or, alternatively, the parasite in focus might be swept up as an innocent bystander in a heterologous, parasite-induced immune response. A good example of the latter was provided by Kennedy (1980b), who studied interactions in the mouse between the intestinal parasites Trichinella spiralis and Nippostrongylus brasiliensis. Kennedy found, in the absence of interspecific competition and antigenic cross-reaction, that non-specific effects of a homologous immune response could affect the heterologous species. Among the effects noted was a change in location in the gut by T. spiralis, an effect which may be considered to be a behavioral change.

SUMMARY

Available evidence suggests but does not prove an influence of host immune response on parasite behavior. Giving free rein to imagination, one can envision various ways in which the immune response could be perceived, directly or indirectly, by parasites and could cause them, directly or indirectly, to modify their behavior. Such changes in behavior could be the result of stress, or could add to stress, and would appear to signal a state or an impending state of physiological distress. Moreover, normal behavioral patterns such as those involving site selection, pairing, and oviposition could be altered, perhaps detrimentally. Such possibilities suggest exciting areas for future research.

ACKNOWLEDGMENT

This work was supported by the United States-Japan Cooperative

Medical Sciences Program administered by the NIAID of the NIH, Department of Health and Human Services (Grant AI 16929).

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DISCUSSION

BENNETT: Concerning your ideas about the tegument, or disruption of tegumental functions alternately leading to reduced fecundity, a couple of years ago we made an epidermal preparation by the method of Oates and Cain. The epidermis was removed from 6,000 male parasites and injected into an isogenetic mouse, and an antibody against this tegumental fraction was developed. We did this experiment twice. We then took the antibody and labeled it with fluorescein or I-125 to make sure they would identify the surface. It seemed to have the proper kinetics. At least it recognized the surface of the Schistosoma. We then asked the question that I was most interested in, "If there is an antibody that recognizes the surface of the Schistosoma, does it express itself in changing the motor activity of the parasite?" And I worked and worked, swamping the thing with mouse serum with complement, incubating it for two or three or four hours before making my recording, and could never really see any significant change in the motor activity of the parasite. And then, of course, we compared this to controlled worms exposed to everything but the appropriate antiserum. And I was rather disappointed. I do know that with respect to this problem of reduced oviposition Nelson recorded in Schistosoma bovis that they tried to take the serum from animals that had a chronic infection where the oviposition for the female had been reduced and to take quite a bit of serum and inject it into a recently exposed animal to see if they could get the transfer of the factor into such an animal and if it would reduce fecundity in a recently exposed animal by transferring this serum. They couldn't do anything.

DAMIAN: I would imagine it would be a very difficult thing to do. I sort of see this as a slow wearing down of the worm.

BENNETT: I mentioned before the work with the monoclonals with so much more drastic effects. We did indeed take the so-called monoclonal antiserum that Phillips developed against tegument,

added it to the culture system, kept the parasite overnight, and then tried to record muscle activity and membrane potential. And there were three or four of them, or maybe even six if I remember right, that did cause significant alteration. I mean it was tremendous, very dramatic, in the behavior of the parasite.

DAMIAN: I meant to ask you--what medium?

BENNETT: RPMI 1640. And we don't reinforce it with any serum because, of course, adding mouse serum on top of something like horse serum might be a ridiculous experiment. We have to use the antiserum only as a source of serum. It doesn't contain any serum components other than the antiserum which we had, and complement. It's not the most ideal situation, but I think maybe with a monoclonal we may be even able to pull something out. But just using the "gemish" of epidermal antiserum directed against epidermal fragments we really didn't see too much. We were hoping we would.

DESPOMMIER: It occurred to me that I have heard recently from Peter Shantz at the CDC about the difference between ocular ascariasis and visceral migrans--caused by the same organism. He's convinced that the difference in those two clinical syndromes is a reflection of the difference of the state of immunity of the host. That would obviously fit into the category of response-behavior change of the parasite, depending on how many parasites were there to begin with. He was convinced the more the parasite numbers, the greater immune response and less the chance for ocular damage, because they would be held in the tissues. I think that's the way he envisions that.

LYLES: Some years ago I did some work on feeding of Toxocara to immunosuppressed dogs. At the time it was felt that the immune response was in some way related to the fact that after one month of age, for example, you do not get the establishment of adult parasites in the intestinal tract of the host. Under one month, of course, you can with the feeding of infective eggs. We were able to establish some of the parasites in the male dog. We did some gonadectomies and were able to reverse this occurrence of the worms in the gut after a certain period. I'm not sure just how much the immune system is involved here. But still, we were not able to establish the worms in the intestinal tract in the female, only in the male. Of course, this could be reversed in the female by giving the male hormone to the female.

DAMIAN: That's something one should look further into.

KEMP: I've got one question for Jim Bennett, and then back to you with some things. When you labeled those worms and started measuring them for electrical difference, how soon did you look at them after you put the antibody in?

BENNETT: We put them in the presence of the antiserum for let's say an hour and then looked at them. Take a sub-population out and look at them an hour, two hours, three hours, four hours, up to about eight hours. And then, of course, we found that the mouse serum itself, as you may know, is lethal to schistosomas grown overnight. It will kill them outright. I don't know why that is. They seem to grow quite happily in the mouse serum when it's in the in vivo stage. But we have consistently found that when you take mouse serum and try to culture schistosomes overnight they die, at least in the mouse serum that we had. So our experiment was rather acute in terms of looking for effects. They were short-term, but with one to eight hours timing. There could be changes that occur later on. Unfortunately, we can't determine what those changes are as a result of the factors in the mouse serum which in vitro caused death to the Schistosoma. And it isn't related to the antiserum, because we used serum from the mice that weren't given the epidermal injection.

KEMP: My suggestion would be that you look earlier--like in 15 minutes. If you are getting antibody binding, sticking to parasite antigens, then what you may be getting is shedding of those antigen-antibody complexes.

BENNETT: They begin to shed off, yes.

KEMP: We tried to get binding with immune serum per se to fresh worms before. And we get a minimal sort of binding. If we use a minimum IgG on worms that have been allowed to lose their host-associated immunoglobulins, we get lots of binding.

BENNETT: What we're careful of, of course, is when we take the worms out, we like to let them sit in the RPMI 1640 for about an hour to shed whatever adhered IgG they may have from the host. And then put them back in the RPMI that contains the antiserum to see if we can more or less optimize the number of receptors that we pick up with the antiserum from the mice that we have injected with the epidermal fraction. But as you know it's hard to do these experiments because it takes 5,000 males, 10,000 altogether, for two experiments. And it takes a while to collect the membranes and do the injections. It took us about a year before we really could come around to even asking many questions because you had to go through this same cycle. I think the use of monoclonals is the way to go rather than with making antiserum in isogenic mice.

KEMP: I'm curious to know if you have any evidence that there is maybe a shift in antigen recognition with longer term chronic infection. Are there new antigens that have immune responses later on?

DAMIAN: There is a shift. But the shift is in chronic infections as compared with acute infections; there's a downshift.

It's very restricted. These restrictions are in what the animals producing antibodies do. This is true I know in various non-human primate and human infections. I'm not sure about whether new things are seen. And I don't know why or even if this restriction in specificity is just apparent rather than real. We're getting ready to examine that with much more sensitive techniques than immunoelectrophoresis. Something is going on there with antibodies. I don't know if it's suppression or what. But I hope that with the recent development of Mishell-Dutton technology with baboon lymphocytes by my student, Mark Powell, we can establish an antibody-producing assay from chronic infections to answer the question if there's suppression or not. Or is it something else like central failure, or some turnoff of antigens by the worms? All these possibilities exist.

KEMP: You might want to comment on this. How about selection of genetic variants to the parasite by the immune response?

DAMIAN: Well, that's certainly a possibility. That's obviously going on in African trypanosomes and malaria. In helminths there is no evidence for antigenic variance that I know of. There is evidence for genetic variance in the Schistosoma cercariae. But it has not been linked to antigenic variation.

KEMP: That could be one modification--antigenic variation--but there could also be other selections that you wouldn't measure necessarily by a change in antigenic composition.

DAMIAN: Castro, do you want to comment about that?

CASTRO: What we thought we might be dealing with was that residual population Despommier had asked about--why it always seemed to be there. We thought it might be the host or that there is a genetic variant of the parasite, certain populations of them, that are able to bypass that rapid-rejection response established in the gut and generate the secondary response to get rid of them. But what we did, leading us to think that that's not the case, was to immunize the rats by giving them repeated short-term infections. Leave the worm in the gut for 24 hours, give them methyridine to terminate the infection, wait a couple of days, and do that two more times. Then you can grow up a very strong immunity from the standpoint of the rat rejection and pass it to animals that elicit that rejection. We then got the immune response again without having the larvae, the L_2 larvae, going into the muscle from the immunizing dose. So when we challenged and got a small population of worms in the immunized animal, then all the worms, the larvae, that we recovered from muscle would be from those worms that were able to establish and bypass the rejection response. We anticipated that if this was a genetic variant, we would get very high infectivity from that population which we took from the muscle and put it back into another immune animal. But

when we did the second experiment, the infectivity rate was no greater than the stock population of the worm. So it didn't look like we were dealing with anything genetically different from that small population in terms of their ability to get by rapid rejection.

I'd like to go on another step here. You're talking about antigenic variation, and there is very little evidence for antigenic variation. Any comments?

DESPOMMIER: The rapidity with which some parasites evolve through their life cycle could reflect any pressure on behavior; I'm thinking now in terms of Trichinella--what else. Because it has such a rapid molting cycle. And it has such a rapid mating courtship and such a propensity for throwing out these newborn larvae in such a very short time as compared to a lot of other nematodes which hang around like this. The slow ones never made it through. They were selected against right away. The result is the world's fastest molting organism living in the gut for a couple of weeks to a couple of months, depending on the immune system of the host you're talking about. I didn't mention yesterday that Trichinella is capable of living as an adult for 83 days in the nude mouse before the nude mouse dies from something else. It tells us that the life span of the worm is totally under the control of the immune system. That's got to be some kind of behavior pressure.

DAMIAN: Now, we are getting to the argument of what you want to call behavior and what you want to call an evolutionary built-in modification of the life cycle.

METTRICK: There is a difference in the cycle of Hymenolepis diminuta infection related to the variety of rats you use. In the case of the Sprague-Dawley, it is three days longer than that in the Wistar rat. The rats eat exactly the same amount of feed. Their rate of growth is actually the same. We've looked at every sort of parameter and still have the fact that it takes three days longer. I don't have the answer as to why that is so, unless there is some genetic difference in the basic metabolism of the Sprague-Dawley which causes an extension of the patency period.

ROBERTS: I'll add one more example which addresses real behavior. And I will make that provocative statement clear in a moment. When I was working with Ralph Thorson some years ago, I worked on pheromone behavior in Nippostrongylus. And one of our ideas was that perhaps we could use the effects of the host immune system to modulate the behavior of the parasite in some way. Our initial approach was that perhaps the immune system would, as you mentioned, interfere with the parasite's ability to perceive the pheromone signal. What turned out to be the case was that the immune system, probably in some very indirect way, damaged the ability of the parasite to produce

the signal. And Leon Bone may want to either add to or subtract from this. The effect was that we took normal worms, those that had not yet begun to be expelled from the intestine, and looked at the sexual attraction pattern and compared that in vitro with the attraction pattern of damaged worms, that is, the residual worms that are left after expulsion has begun. We found that damaged females produced less pheromone than normal females. And the controls were that those damaged females were quite capable of responding to a pheromone source for normal worms. And the other control was that if we took worms that were 14 days old that had been manipulated to avoid the immune response by allowing them to grow 7 days in one rat and then transferring them to a new rat every two days so they are never affected by immunity--at least not a great extent--the transferred worm showed exactly the same behavior pattern as normal worms. So the effect was not one of senescence, but rather one of host immunity. And I don't know that that's ever been followed up.

BONE: Basically what you have said I accept. I think the only modification I would make is putting it in Mike's 22 analogy that the male is also subjected to it. But it's more or less a hit or miss phenomenon when you look at a population level. Some appear to be blocked, perhaps for reception, while others are untouched; or perhaps there are degrees of blocking for any individual.

FROM RECEPTOR TO RESPONSE: PROSPECTS FOR RESEARCH IN BEHAVIORAL PARASITOLOGY

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INTRODUCTION

Tremendous advances have been made in the past few years in understanding behavioral mechanisms in microorganisms such as bacteria, Dictyostelium, Paramecium, Chlamydomonas, leucocytes, and Caenorhabditis. In comparison, the field of behavioral parasitology is in its infancy. There are, no doubt, several reasons. For example, the discipline covers several topics (host finding, host entry, migration, site selection, mate location, etc.), and there is certainly no lack of interesting organisms to work on (see, for example, Compton 1976). As a consequence, "critical masses" of investigators have not accumulated to attack specific problems. A second reason, probably of greater importance, is that much of the behavior of parasites takes place in the relatively inaccessible and complex environs of host tissue, the conditions of which are difficult or impossible to mimic. Compounding this problem, from the viewpoint of both the parasite and the researcher, is the host's tendency to alter its physiology (see paper by Castro, this volume) and to mount an immune response (see paper by Damian, this volume) in the face of infection. Nevertheless, as the preceding papers in this volume attest, many ingenious approaches toward studying parasite behavior have been devised so that we continue to progress, often via surprising and interesting results.

Suggesting pathways for future research in such a young field as behavioral parasitology is a difficult task because so much lies ahead. Behavioral systems in general comprise three parts: a sensory mechanism by which the stimulus is detected, a transduction mechanism which transforms sensory information into intra- or intercellular messages and an effector mechanism by which a response is made (Zigmond 1978). In studies of behavior of other microorganisms, such as those listed above, sensory mechanisms are usually examined first, followed, in

order, by effector and transduction mechanisms. Because most current work on parasite behavior addresses sensory mechanisms, it seems logical that our path will follow the course taken by our colleagues studying behavior in other microorganisms. Thus, I will use the three components of behavioral systems as a framework for presenting some thoughts about the possible future directions of behavioral parasitology. At the outset, I admit a bias toward chemically-mediated behavior. I do so simply for convenience and acknowledge that many other types of stimuli (e.g., light, heat, gravity, touch, etc.) influence parasites, especially when larval stages seek hosts (see, for example, Thorson 1969, Cable 1972, Croll 1972).

AN OPENING WORD OF CAUTION

Our fascination with the behavior of parasites derives, in large measure, from the complexity of their life cycles. The barriers that parasites overcome in finding hosts and the intricate migrations they undertake between host entry and establishment in preferred sites have stimulated our imaginations. In our zeal, we have tended to ascribe every turn of the worm (or protozoan) to some behavioral cue. Austin MacInnis (1976) has presented a delightful conceptual treatise on parasite behavior. His scheme divides the parasite's role in the host-parasite interaction into active (parasite is cued by the host) and passive (chance encounter) modes. We will, no doubt, find that some of the parasite movements now considered active will turn out to be passive when examined closely. An example is Croll's work (Croll 1976, 1977, Croll and Ma 1978) on the movements of Nippostrongylus through the tissues of the rat. He showed that much of the intricate larval migration in this species was due to passive transport through the host's "pipes and tubes" and not to directed responses to host-derived stimuli. The implicit message from Croll's work is that we can find several clear-cut cases of active parasite responses to work on without seeking them where they do not exist. Neil Croll was a guiding voice in behavioral parasitology; we will miss him.

SENSORY MECHANISMS

Sensory mechanisms center on the binding of chemical stimulants to their receptors. These receptors are responsive not to the absolute concentration of bound receptor molecules but rather to changes in the percentage of those molecules bound to stimulants (for a thorough discussion see Brown and Berg 1974, Mesibov et al. 1973, Ward 1978). Therefore, behavioral responses are generated by gradients of chemical stimuli rather than simply by the presence of those chemicals. As a result, the analysis of parasite sensory mechanisms involves several questions: can gradients form in the turbulent environments occupied by parasites? what are the stimulant molecules? what are the receptors and where are they located? how do receptors detect changes in stimulant concentration?

Establishment of gradients by diffusion from a source requires both time (related to the diffusion coefficient of the molecule) and enviromental stability. These are not outstanding features of the habitats occupied by parasites, yet we now have several examples (see papers by Mettrick, Bone, and Dusenbery, this volume) of chemically-mediated parasite behavior in vivo. There are several possible solutions to this dilemma. One is that responses may be kineses (changes in rates of movement or turning) rather than taxes (directed movements). This type of response requires only that the parasite detect the proper place to be and stay there rather than moving over long distances to a discrete goal. Schistosoma mansoni miracidia respond to a miraxone gradient by chemokinesis (in this case, by turning back when they detect decreases in stimulant concentration) rather than chemotaxis (Roberts et al. 1979). Other parasites being passively carried through the host's plumbing or wandering through the tissues could use this same mechanism to select their desired sites. The advantage (to the parasite) is that the gradient need form over only a short distance.

Another variation on this theme is that directed responses could be made to short gradients. An example may be mate location. Bone and coworkers (see paper this volume) have shown that Nippostrongylus adults make directed movements in gradients and that they can, to some extent, discriminate between sexes chemically. Once a population of adults has reached the small intestine a worm may need to follow a gradient to a mate located only a few millimeters away.

Gradients would be stabilized in turbulent environments if guidance cues were bound to particular channels or pathways. This phenomenon may guide some cell migrations during embryogenesis of multicellular organisms (reviewed by Katz and Lasek 1980). We have no evidence of this occurring in parasite migration, but intracellular protozoa may recognize their host cells by a simplified version of this mechanism. It is reasonable to predict that complementarity between surface molecules may enable protozoans to recognize their host cells. Precedents are found in interactions between eukaryotic gametes (Wiese and Wiese 1978), and we know that Duffy blood group determinants on erythrocyte surfaces may be involved in cell recognition by Plasmodium merozoites (Miller et al. 1975). We need to carry the analogy only one step further for parasitic metazoans to detect guidance cues bound to the surfaces of cells lining a migratory pathway.

A survey of chemical attractants detected by the microorganisms which I have presented as our "models" suggests that a variety of types of substances may be involved (sugars, nucleotides, peptides, inorganic ions, and glycoproteins). One generalization, which remains to be proven, is that stimulants of parasitic behavior may be small molecules which are able to diffuse rapidly (the glycoproteins mentioned above function in

cell-cell recognition and, therefore, need not diffuse). Otherwise, we are in for some lengthy detection work taking apart mixtures of molecules and assaying to find the active components. The most rapid progress may come in identifying pheromones (because here, at least, the source can be defined). Fried and co-workers (Fried and Gioscia 1976, Imperia et al. 1980) have implicated sterols as pheromones for several digenetic trematodes and Bone and colleagues (Bone et al. 1979, Bone et al. 1980) are well on their way toward identifying aggregation and sex pheromones emitted by Nippostrongylus. A lesson should be learned from Mettrick's group (see paper this volume) who parlayed an understanding of parasite behavior and host physiology to deduce that 5-HT influences diurnal migration of Hymenolepis in the small intestine.

Locating and identifying receptors for these ligands may be tricky. Metazoan parasites seem to be richly endowed with presumptive sensory neurons (see, for example, Dusenbery, this volume). But because these cells are small and relatively inaccessible they are difficult to isolate or to penetrate with electrodes. Thus, it is difficult to assign specific chemical sensitivities to specific neurons. The genetic tricks that can be played with C. elegans have helped sort these assignments out to some extent (Ward 1977, 1978, Dusenbery, this volume), but even there mutants with specific behavioral lesions do not always show corresponding specific neuroanatomical defects.

Organisms use either a spatial or temporal mechanism to detect changes in stimulant concentration within a gradient (see Zigmond 1978). In the spatial mechanism two or more receptors at different locations compare simultaneously the stimulant concentration (probably by comparing the ratio of bound-to-free receptor molecules at the two sites) in order to determine the direction of the gradient. In the temporal mechanism concentrations are compared by the same (or, conceivably, different) receptors at successive times (again, by measuring the percent of bound receptor molecules). Small organisms can span only a minute fraction of a gradient and would need to be sensitive to very small changes in stimulant concentration to use a spatial mechanism. Thus, it seems reasonable that they would use temporal detection mechanisms (plus an ability to move relatively long distances through the gradient) to orient. This is the case for bacteria (MacNab and Koshland 1972, see Brown and Berg 1974 for an elegant discussion of why this must be true) and probably leucocytes as well (Zigmond 1977, but see also Zigmond et al. 1981). Helminths would seem to have overcome the size restrictions imposed on spatial detection. Nevertheless, the chemoreceptors for several classes of attractants for C. elegans (see Dusenbery, this volume) are located exclusively in the head. Ward (1978) has analyzed the movements of the head of worms moving in a gradient to develop a model for temporal detection in this comparatively large animal. We may find that the trematodes, like nematodes, fail to take

advantage of their long anterior-posterior axis. The sensory neurons of miracidia (Pan 1980) are arranged radially or bilaterally rather than longitudinally, and those of adults may be arranged in the same way.

If one set out to design an efficient spatial detector the result would probably look much like a cestode--a long chain of cells, each capable of detecting a stimulant, attached end-to-end so that the sensors spanned a large fraction of the gradient. Evidence is already accumulating that Hymenolepis uses a spatial mechanism to detect a 5-HT gradient in the gut (Hopkins and Allen 1979, Mettrick, this volume) presumably by integrating input from proglottids along the length of the worm which are assaying simultaneously the 5-HT concentration at their particular locations.

EFFECTOR MECHANISMS

Most of the responses of parasites to behavioral cues will probably involve directed motility. We already know, at least superficially, how most parasites move. To understand how movement is directed we need to do some careful analyses of parasites moving in gradients in vitro. Among the metazoans, there is evidence that the anterior end of the animal serves as a rudder so that anterior tip orients to the gradient and the musculature provides propulsion. Ward (1973) used an ingenious genetic trick to show that the head of C. elegans functions as a rudder. He tracked mutant worms with bent heads in chemical gradients. The head oriented to the gradient but because of the bend the propulsion generated by the body wall muscles pushed the animal in a series of loops. A lengthy series of reorientations eventually brought the mutant worms to the top of the gradient but their tracks were considerably more tortuous than the relatively straight paths taken by wild-type worms. We might expect that trematodes and cestodes also use their anterior ends as rudders by pointing in the right direction, attaching, and pulling the rest of the body forward.

A different type of effector mechanism currently receiving considerable attention is the mechanism of host cell entry by intracellular parasites. (I counted 11 papers in the past three years on various protozoans and I probably missed a few.) The subject strikes at the heart of many of the most important parasitic diseases (malaria, leishmaniasis, Chagas' disease) and, thus, has a built-in importance. But of equal significance, we are dealing with a matter of interest to the biological community at large--namely, the area of cell-cell interaction and the ways one cell directs the activity of another. I allude to the theory that the parasite induces its own phagocytosis by the host cell and, as a consequence, forms that mysterious organelle, the parasitophorous vacuole. No doubt, there are numerous molecules communicating back and forth between parasite and host. With the proper approach (see below,

for one possibility) we should be able to identify these molecules and understand their functions.

TRANSDUCTION MECHANISMS

Sensory transduction (the translation of receptor input into effector output) can be examined at the molecular or the cellular (neuronal) level, depending in large part on the organism being studied. Work on unicellular organisms has demonstrated a few key molecules that function in sensory transduction in several different types of cells. For example, protein carboxymethylation plays a role in transduction during bacterial (Kleene et al. 1979, Kondoh et al. 1979) and leucocyte (O'Dea et al. 1978) chemotaxis with s-adenosylmethionine serving as a methyl donor. Ca^{2+} fluxes (and presumably also calmodulin) are important in a variety of ways in several different cells (Ordal 1977, Kung et al. 1973, Naccache et al. 1979, Zigmond 1978). Not surprisingly, cyclic nucleotides also play a role (Black et al. 1980, Mato et al. 1977). This list is not inclusive (the interested reader is referred to the cited papers for details). There is not a single behavioral system where the entire transduction mechanism is known, although bacterial chemotaxis is close to becoming an exception. I present these examples as possible starting points when we begin to look at molecular transduction mechanisms in parasite behavioral systems. An interesting point emerges from Mettrick's (see paper this volume) work on Hymenolepis. There, 5-HT may serve as both an attractant and a transduction molecule for the worm via its effects on glucose absorption, carbohydrate metabolism and neurosecretion. If so, the worm's multiple uses for host-derived 5-HT would be an excellent example of the evolutionary parsimony of parasitic animals (Thorson 1969, MacInnis 1976).

At the cellular level, transduction becomes an analysis of neuronal interconnections seeking to understand how sensory information is fed into a central nervous system (or its equivalent), integrated, and then fed out to appropriate motor neurons. As discussed above, pinpointing the function of sensory neurons in micrometazoans is a difficult task, so taking apart the entire neuronal system becomes near impossible. Even in C. elegans, where most of the neuroanatomy is known at the ultrastructural level (Ward et al. 1975, Ware et al. 1975) and numerous behavioral mutants are available, functional neuronal connections are not understood. Yet, there are reasons for hope. The neuroanatomy of Ascaris is similar to that of C. elegans, and the larger size of the parasitic worm allows the biochemistry and electrophysiology of individual neurons to be studied (Johnson and Stretton 1980) and correlated with the genetic analysis of C. elegans.

PROSPECTS FOR GENETIC DISSECTION OF PARASITE BEHAVIOR

A common feature of the analyses of behavior of model micro-

organisms is the value of induced mutations in dissecting the details of sensory, effector, and transduction mechanisms. This has been true for gametic interactions in Chlamydomonas (Goodenough et al. 1976), chemotaxis and aggregation in Dictyostelium (Newell 1978), swimming behavior and chemotaxis in Paramecium (Eckert 1977) and, as discussed above, chemotaxis in C. elegans (see Dusenbery, this volume). Genetic analysis of behavior reaches its pinnacle in bacterial chemotaxis (Parkinson 1977) where mutants have been indispensable tools for unraveling the details of each of the three components of the behavioral system. The approach has been so thorough that bacterial chemotaxis can now be thought of as a biochemical pathway.

I suggest, by analogy, that if we are to understand the molecular details of parasite behavior we will need to apply genetic techniques. Once organisms amenable to genetic analyis have been chosen, we need only induce mutations, devise techniques to screen for the phenotypes of choice, clone and propagate the mutant strains, and (if possible) work out reproductive techniques for making genetic crosses (the last is worthwhile, but not absolutely necessary).

I do not mean to imply that mutant analysis should be a panacea for solving all problems of parasite behavior. The genetic task is an arduous one and should be undertaken only when a tractability for genetic analysis and background data on interesting behavior coincide in the same organism. Perhaps a prime set of candidates for developing a genetic system for studying parasite behavior would be apicomplexans such as the coccidia Plasmodium and Toxoplasma. These can all be grown in tissue culture in quantities sufficient for biochemistry, and Pfefferkorn and Pfefferkorn (1976) have already devised techniques for isolating and characterizing mutants of Toxoplasma. The particular advantage of this group is that for most of their life cycle they are haploid and will multiply without going through a diploid stage. Thus, recessive mutations will be expressed phenotypically without resorting to brother-sister mating to create homozygotes. Haploidy here creates a disadvantage because mutations which alter the host-parasite interaction will probably be lethal. This can be overcome by studying temperature-sensitive mutants which express the mutant phenotype at a restrictive (usually higher) temperature but which exhibit the wild-type phenotype (allowing strains to be maintained) at the permissive temperature. There is even potential (for Toxoplasma at least) of doing crosses because Pfefferkorn et al. (1977) have shown that cloned trophozoites produce both micro- and macrogametes when fed to cats. That the products of each gametic interaction emerge from the cat intestine enclosed in an oocyst adds another feature to the amenability of this organism to genetic study because the progeny of each cross can be isolated and analyzed.

Collection of behavioral mutants of these organisms should open up avenues for examining particularly interesting features of their interactions with their host cells. For example, Toxoplasma undergoes at least three or four different cell-cell interactions during its life cycle (trophozoite with several types of host cells, trophozoites with feline intestinal cells, microgametes with intestinal cells harboring macrogametes, microgametes with macrogamates). Mutants would assist the study of each of these as well as the developmental regulation of the interaction molecules as the parasite proceeds through its life cycle. Another example is Plasmodium. Here, parasite mutants combined with the existing knowledge of genetic determination of blood group antigens on erythrocytes would allow both sides of the cell-cell interaction to be approached genetically.

An underlying theme of this workshop has been that studying parasite behavior should allow schemes to be devised to use behavioral confusion as a means of biological control. This is, indeed, a lofty pursuit worthy of our efforts. To that end, I have tried to point out leads available from research on the behavior of other microorganisms which should assist us in obtaining the information we need to apply our research to the parasites' demise. But, another purpose should be kept in mind. The parasitic lifestyle is unique and, as a result, we should be able to choose parasites which will serve as ideal models for studying particular behavioral phenomena. Balanced polymorphism between malaria and sickle cell disease, molecular mimicry in schistosomes, and antigenic variation in trypanosomes come to mind as examples from other areas of parasitology which have achieved this status in population genetics, immunology, and developmental biology. As suggested above, the interplay between intracellular protozoa and their host cells may be ready to make important contributions to cell biology and behavior, and I am confident that other similar examples will develop as we learn about the basics of parasite behavior. The key here is that we stand at a powerful position of being able to make valuable contributions to both applied and basic research.

ACKNOWLEDGMENTS

I thank Jenny Britton for preparing the manuscript and my colleague, Bob Short, for critical reading of the text and many hours of valuable discussion of the prospects of genetic manipulation of parasites.

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DISCUSSION

LONG: We have addressed the problem of how Eimeria sporozoites recognize their host cells by receptor-ligand complementarity. Do you have to postulate that all host cells have the receptors for their Eimeria, or do you postulate that none of them have receptors? Or can you postulate a third possibility? These host cells--this just occurred to me--are recovered by trypsinization. Then we have to postulate that perhaps the receptors are removed by trypsinization.

ROBERTS: We should, perhaps, think of the parasite-host cell interaction in more general terms. Very few of the coccidia you showed in your presentation were outside their host cells. So it's obviously important to the coccidia to be inside the cell. We tend to take an anthropomorphic view of parasites. We tend to think that if there's a cell that we can see under the microscope, then the parasite can certainly see it. But the parasite can't see it. And they have to find some way of detecting where a cell, any cell, is and determining how to enter it. Another example is the Toxoplasma trophozoite which can invade just about any cell, if you want to look at parasite cell specificity.

LONG: It's the same idea. Eimeria and Toxoplasma are very closely related actually.

ROBERTS: There may be instances where there is greater specificity, such as the relationship between Toxoplasma trophozoites and feline intestinal epithelial cells. Another possible receptor-mediated interaction may be the mechanism coccidian microgametes use to detect host cells harboring macrogametes. Do macrogametes change the surface of the host cells so that microgametes can recognize them? Apparently Plasmodium merozoites residing in red blood cells alter the antigenic nature of the host cell surface. Perhaps the same sort of thing occurs in the coccidia. I am interested in your comments.

LONG: Yes. I think the specificity in the coccidia lies very much more in the completion of the life cycle, as I pointed out yesterday. Foreign hosts can often support a certain degree of the parasite's sexual cycle. But the parasites do not complete the life cycle. That end of the cycle is paramount. In regards to the changes in the host cell harboring the macrogametes, I think there is some evidence that we have a permeability change when cells are penetrated. The entire cat intestine has a change of permeability associated with sporozoite infection. There is a great vascular change with serum proteins poured out into the gut in response to the sporozoite. We have another problem, as you mentioned. That is, how the microgamete finds the macrogamete and how it enters that cell seems very strange to me. The microgamete must pass through two cell membranes to penetrate the macrogamete. This is another problem I'd like to understand, for the receptor phenomenon would be very much more alert in that situation.

DESPOMMIER: It's obvious that this discussion can continue because a lot of parasites are able to enter and exit the cells. Obviously Trichinella does the same thing. Once it gets into the right cell, it stays there. That's another set of behavioral cues. You've got to talk about this because the movie shows coccidia going in and out of host cells like crazy. They obviously were not in the right cell or they would have stayed inside. Gil (Castro) would suggest that the columnar epithelial cell of the small intestine is paramount to understanding a lot of these issues. I believe that, too. I truly believe that, at least in terms of the gut infection that you were describing, you are going to have to start catheterizing.

Comparing the behavior of Toxoplasma and Trichinella in host cells, why does Toxoplasma go to all those other types of cells? And why does Trichinella go into some types of cells and then back out? Trichinella enters and leaves even cardiac muscle cells. It doesn't stay in any type of muscle cell except the striated cell. What is the signal or signals that allows it to stay there? That is an intriguing idea. Why is Toxoplasma non-discriminative?

ROBERTS: I pointed out in one of the discussions here yesterday that we tend to try to reduce these things to one molecule

eliciting one sort of behavior. But when we look at the entire behavioral repertoire of an organism, its interaction with the host may be cued by a number of molecules or perhaps even other sorts of senses. It's that interplay that generates specificity. We have to broaden our thinking and look at complexes of molecules and how they interact in sequence and how they are regulated.

KEMP: It seems to me at its most basic level there has to be some sort of recognition and acknowledgment of the parasite in itself that is programmed as a parasite. A malarial parasite enters the host and bumps into leucocytes, bumps into epithelial cells. Somehow or other, sporozoites recognize the liver and set up shop there. They don't pick the kidney or the brain. That implies recognition.

Now, in terms of receptor molecules, sometimes I get confused when I use receptor molecules when I really mean recognition molecules. So perhaps in some cases they are the same. When you initiate B-cell activation, you do it with three cells. You have the B-cell, T-cell, and macrophage. You just can't take any cell from any host and put them together and do that. There are recognition molecules that have to be there before the activation molecule can activate. So maybe what we've got is a multi-receptor situation where recognition occurs, then penetration and so on.

The film that we saw yesterday was really remarkable. And in watching those organisms play inside those cells, I was reminded of children in a playground. It was as if they didn't really live there. And they weren't particularly happy staying there all the time. But they were swinging on the swings and sliding on the slides and having a grand time coming out to the other side and sitting there and talking and saying, "Maybe I'll rest a little bit, and maybe I'll do it again." And in the host obviously that doesn't happen. They find a home and stay there. As Dickson (Despommier) said, perhaps there are signals for a parasite to enter a cell, and other signals for determining whether to stay in that cell. Thus, there may be a biphasic recognition system.

ROBERTS: Every point that you added makes it more interesting, at least in my mind. Conversely, it also makes it harder to understand.

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☆U.S. GOVERNMENT PRINTING OFFICE: 1982—569-036/30C

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